3/5/97-01382

Draft

Treatability Study Report on Explosives - Contaminated Soil Naval Weapons Station Yorktown Yorktown, Virginia

Prepared For

Department of the Navy Atlantic Division

Naval Facilities Engineering Command

Norfolk, Virginia

Under The

LANTDIV CLEAN Program

Comprehensive Long-Term Environmental Action Navy

Reference:
Contract
N62470-89-D-4814

CTO-0209

March 1997

Baker

3/5/97-01382

DRAFT

TREATABILITY STUDY REPORT ON EXPLOSIVES-CONTAMINATED SOIL

NAVAL WEAPONS STATION YORKTOWN
YORKTOWN, VIRGINIA

CONTRACT TASK ORDER 0209

MARCH 5, 1997

(FINAL VERSION OF DOCUMENT)

Prepared for:

DEPARTMENT OF THE NAVY
ATLANTIC DIVISION
NAVAL FACILITIES
ENGINEERING COMMAND
Norfolk, Virginia

Under the:

LANTDIV CLEAN Program
Contract N62470-89-D-4814

Prepared by:

BAKER ENVIRONMENTAL, INC.
Coraopolis, Pennsylvania

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LIST OF ACRONYMS AND ABBREVIATIONS

1,2-DCE	1,2-dichloroethene
1,3,5-TNB	1,3,5-trinitrobenzene
Baker	Baker Environmental, Inc.
DoN	Department of the Navy
FS	feasibility study
HMX	1,3,5-tetranitro-1,3,5,7-tetrazocine
IRRP	Installation Restoration Research Program
mg/kg	milligrams per kilogram
RDX	hexahydrotrinitro-1,3,5-triazine
SERDP	Strategic Environmental Research and Development Program
TAL	Target Analyte List
TCE	trichloroethene
TCL	Target Compound List
TNT	2,4,6-trinitrotoluene
TS	treatability study
µg/kg	micrograms per kilogram
USAE	United States Army Corps of Engineers
VOC	volatile organic compounds
WES	Waterways Experiment Station
WPNSTA	Naval Weapons Station

1.0 INTRODUCTION

Past weapons loading and packing activities at the Naval Weapons Station (WPNSTA) Yorktown, Yorktown, Virginia (also referred to as the Station) have resulted in the contamination of soil with explosives compounds such as 2,4,6-trinitrotoluene (TNT), hexahydrotrinitro-1,3,5-triazine (RDX), and octahydro-1,3,5-tetranitro-1,3,5,7-tetrazocine (HMX). The Department of the Navy (DoN) has been assessing the extent of explosives contamination at the Station and evaluating various remedial options. As part of a remediation assessment, the DoN tasked the United States Army Corps of Engineers (USAE) Waterways Experiment Station (WES), Vicksburg, Mississippi to perform a bench-scale treatability study (TS) on various candidate biological treatment processes for explosive-contaminated soil from the Station. Additionally, this work was part of the Strategic Environmental Research and Development Program (SERDP), Installation Restoration Research Program (IRRP) and the U.S. Army Environmental Quality Technology Research Program.

1.1 Objective of the Treatability Study

The overall objective of the TS was to evaluate three soil treatment techniques (anaerobic biotreatment, aerobic biotreatment, and slurry oxidation treatment) for potential application of the WPNSTA Yorktown soil. The results of the TS were to be used by Baker Environmental, Inc. (Baker), the DoN's contractor, in the preparation of feasibility studies (FSs) for several WPNSTA Yorktown sites.

1.2 Treatability Study Outline

WES prepared and submitted a final work plan for the TS in May 1995. The work plan included a seven-phase approach outlined below.

- Phase I - Soil Sample Selection and Preparation

Phase I involved the selection of an appropriate soil sample for the biotreatment tests and the logistics of shipping and storing the sample. This phase included the following five subtasks:

- Task I-1 Selection of a Soil Sample
- Task I-2 Collection, Homogenization, and Shipment of the Sample
- Task I-3 Soil Sample Storage
- Task I-4 Laboratory Homogenization and Sieving of Soil
- Task I-5 Chemical and Physical Characterization of the Soil Sample

- Phase II - Microbial Systems Evaluation

Phase II included the selection of treatment conditions that were to be evaluated during the TS. The condition selections were based on an assessment of required microorganisms for complete explosive degradation, an evaluation of potential cometabolites, and a determination of an appropriate range of nutrient levels. Phase II included the following four subtasks:

- Task II-1 Assessment of the Explosive-Degrading Potential of Native Yorktown Soil Microflora
- Task II-2 Selective Enrichments of TNT-Degrading Microorganisms From Yorktown Soil
- Task II-3 Assessment of the Efficacy of Adding Exogenous Microorganisms to Bacteria to Contaminated Yorktown Soil
- Task II-4 Evaluation of the Effects of Adding Tween 80 to Yorktown Soil During Biotreatment

- Phase III - Desorption Enhancement Evaluation

The benefits of adding a surfactant for increased solubilization rate of the explosives was evaluated using equilibrium batch and sequential batch leach tests. Tween 80, a commercially available non-ionic surfactant, was one surfactant evaluated in Phase III. This phase was conducted in two subtasks.

- Task III-1 Selection of Surfactant Dose
- Task III-2 Sequential Batch Tests

- Phase IV - Bioslurry Bench Studies

Phase IV included the bench-scale portion of the TS in which both aerobic and anaerobic conditions were evaluated using five-liter, glass bioslurry bench reactors. The treatment conditions used in the bioreactors were determined based on the results of Phases I through III. Phase IV was conducted by the following two subtasks:

- Task IV-1 Aerobic Bioslurry
- Task IV-2 Anaerobic Bioslurry

- Phase V - Biocell Bench Studies

Phase V included the bench-scale portion of the TS in which aerobic and anaerobic conditions were evaluated using one-gallon, bench-scale biocell reactors. The treatment conditions used in the bioreactors were determined based on the results of Phases I through III. Phase V was conducted by the following two subtasks:

- Task V-1 Aerobic Biocells
- Task V-2 Anaerobic Biocells

- Phase VI - SlurOx Bench Studies

Under Phase VI, the potential for using the SlurOx (slurry oxidation) process for treatment of the explosive-contaminated soil was to be evaluated.

- Phase VII - Report

Phase VII includes WES's reporting of the TS. WES has recently prepared a three-part report to document the TS results. These reports have been included in Appendices A, B, and C to this TS Report. WES's reports are currently in the draft stage, and as such WES's author has requested that the reports not be cited. The final versions of WES's three-part report will be included within this TS Report once available.

1.3 Report Organization

This document is organized into three additional sections and three appendices. Section 2.0 presents background information on the biotreatment processes evaluated under the TS. Section 3.0 presents a summary of the activities conducted under Phase I of the TS. Baker was directly involved with the initial activities under Phase I. WES was directly involved with the activities under the remaining phases of the TS, and therefore, Phases II through IV are acknowledged in Section 4.0. A complete discussion of the activities and results from Phases II through VII are presented in WES's three-part report included as Appendices A, B, and C of this report.

2.0 BIOTREATMENT PROCESSES BACKGROUND INFORMATION

Biotreatment processes use enzymatic mechanisms catalyzed by microorganisms to break-down organic compounds. These processes have been widely applied for treatment of municipal and industrial wastewater and groundwater treatment. Recent developments in both bioreactor design and microbiology have allowed biotreatment to remediate contaminated solids (soils, sediments, and sludges). The TS for the WPNSTA Yorktown soil investigated two biotreatment approaches, aerobic and anaerobic, using two biotreatment application scenarios, bioslurry and biocell. Background information on these approaches and scenarios are briefly discussed below.

2.1 Biotreatment Approaches: Aerobic and Anaerobic

2.1.1 Aerobic Biotreatment

Aerobic microorganisms require oxygen as a terminal electron acceptor during respiration and for biosynthesis of fatty acids. Organisms utilizing organic compounds as electron donors are referred to as heterotrophs, while those obtaining all of their energy from sources other than organic compounds are termed autotrophs. Many bacteria, and most fungi, algae, and protozoa are obligate aerobes (i.e., they require oxygen for growth). Lack of sufficient levels of oxygen in a medium can often be responsible for poor growth of aerobic microorganisms. This can be brought about by a number of different factors including poor mass transfer, high abiotic oxygen demand, and limited on-site oxygen production capacity.

2.1.2 Anaerobic Biotreatment

Anaerobic microorganisms utilize biochemical reactions where oxidized compounds serve as electron acceptors and are reduced. This process is fueled by the oxidation of organic or inorganic compounds. In natural systems, reduction of inorganic compounds follows a step-wise sequence predicted by thermodynamics. Once almost all dissolved oxygen has been utilized, facultatively anaerobic bacteria, capable of growth in both aerobic and anaerobic environments, take over from aerobic microorganisms, and other electron acceptors are used in place of oxygen. Initially, nitrate is reduced when all nitrate supplies are consumed, manganese IV is reduced, followed by iron III,

sulfate, and then carbon dioxide. Most obligate anaerobes use organic materials to produce carbon dioxide and methane. Some are extremely intolerant of oxygen.

2.2 Biotreatment Application Scenarios: Bioslurry and Biocell

As previously mentioned, the two biotreatment application scenarios evaluated during this TS were bioslurry and biocell. These scenarios differ from each other in terms of the level of mixing obtained within each system. Bioslurry represents the highest level of mixing available, while biocells are static systems. Mixing represents one of the most costly portions of process unit costs. Therefore, the rationale for evaluation of two reactor configurations is the potential difference in treatment costs that may be realized by WPNSTA. Bioslurry systems are estimated to cost between $90 to $200 per cubic yard treated depending on the removal kinetics obtained and the amendment doses required. Biocells are estimated to cost between $20 to $100 per cubic yard treated also depending on removal kinetics and amendment requirements. Both of these scenario are briefly discussed below.

2.2.1 Bioslurry Biotreatment Scenario

Bioslurry treatment of contaminated soil is a relatively new treatment technology for the destruction of biodegradable contaminants sorbed to soil particles and/or in solution. It is similar to other soil and sludge biotreatment technologies in terms of microbiological interactions and contaminant degradation pathways. However, it differs from the other technologies, because bioslurry systems are capable of substantially increasing the degradation rate of contaminants by increasing the availability of contaminants, electron acceptors, nutrients, and other additives to the microbial consortia. This is accomplished by completely mixing the soil in a water slurry (typically at 40 percent solids); thereby, reducing mass transfer limitations associated with the biotreatment of soil contaminated with hydrophobic contaminants having high sorption coefficients.

For aerobic systems, oxygen levels are maintained by diffusion of air or oxygen into the soil/water slurry. Field screening of the untreated soil is often required to remove large debris and gravel from the soil prior to bioslurry treatment. Bioslurry systems are typically operated in the batch or semi-batch mode. There are a variety of dewatering systems that may be used to effectively dewater the treated soil such as sludge drying beds and filter presses.

Some factors governing the availability of contaminants to microorganisms in a bioslurry reactor are not well understood. However, factors known to influence availability include the aqueous solubility of the contaminant and the rate of diffusion/mass transfer of the contaminant from soil solids to the aqueous phase. Aqueous solubility and mass transfer can be increased by the addition of a surfactant which lowers the surface tension of the soil/water slurry. Explosives compounds have low solubility limits in aqueous solutions due to their relatively neutral polarity. Surfactants may provide a means of overcoming solubility limitations. Based on the positive aspects of surfactant addition in other biotreatment studies, the feasibility of surfactants was evaluated as part of this TS.

2.2.2 Biocell Biotreatment Scenario

Biocells are an economically attractive, biotreatment process design for remediation of contaminated soil. The technology, which involves excavation of the soil, screening to remove larger debris, then loading into the biocells, is best described as "bioventing in a can." Once the soil is loaded into the biocell, little or no mixing is provided. A vertical auger mounted from above the cell may be used for periodic mixing.

Biocells are operated in a true batch mode much like composting. The soil is added into the biocell without slurrying like the bioslurry process. Instead, the soil is simply dumped into the cell and then aeration is initiated to stimulate the aerobes. In some cases, if the soil has a very low hydraulic conductivity, sand or other bulking agents may be added. Low hydraulic conductivity hinders transport of air (which supplies the oxygen) and water (which supplies the moisture and amendments).

3.0 TREATABILITY STUDY PHASE I SUMMARY

Baker was involved in the completion of the initial activities under Phase I - Soil Sample Selection and Preparation. This phase of the TS involved the selection of an appropriate soil sample for the biotreatment tests and the logistics of shipping and storing of the soil sample. Soil sieving and characterization activities were also included under this phase. Phase I consisted of five subtasks which are discussed below.

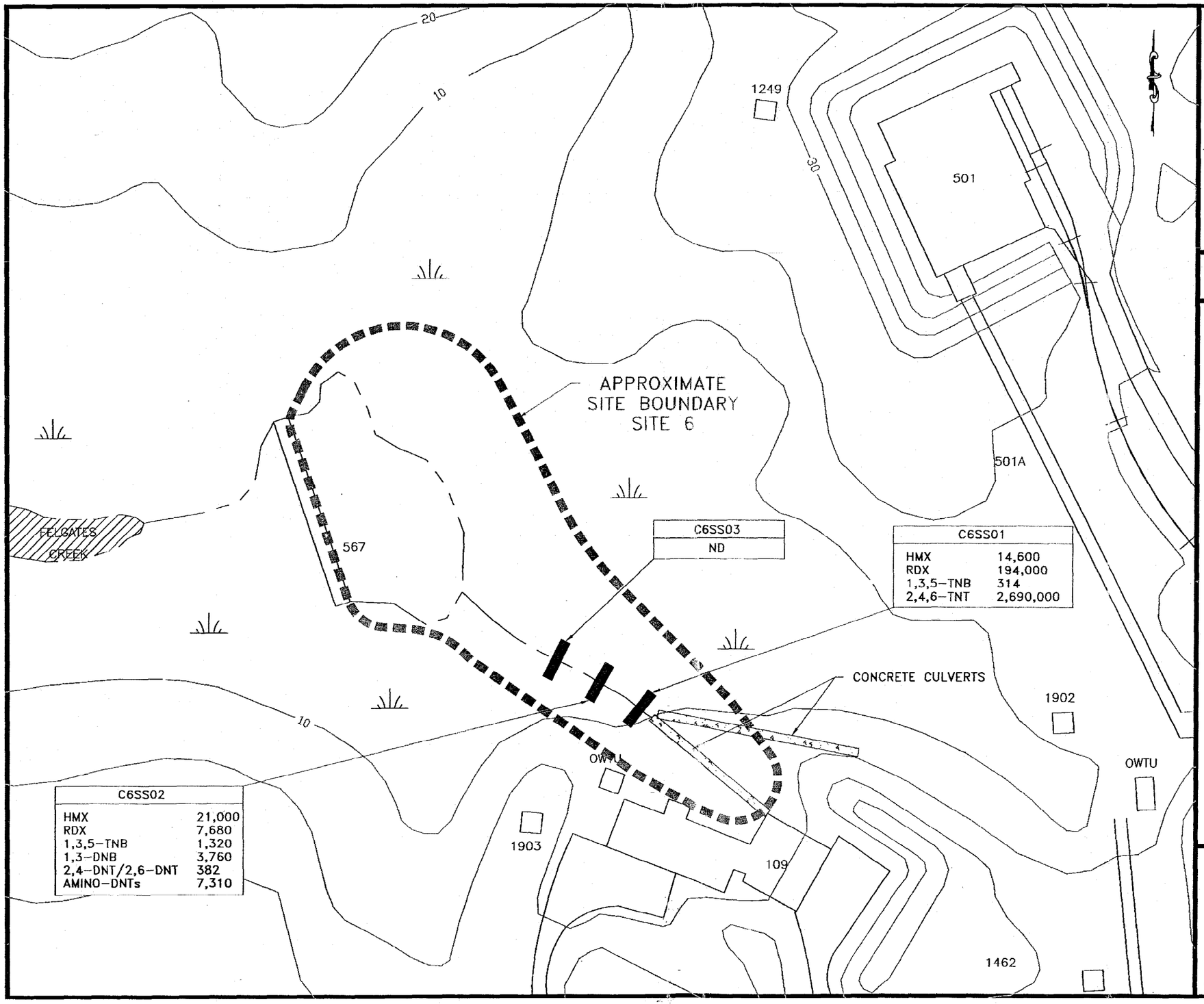
3.1 Task I-1 - Selection of a Soil Sample

Task I-1 (Selection of a Soil Sample) included the collection and evaluation of soil characterization data prior to the selection of the treatability study soil sample.

Soil characterization data was collected by Baker from December 6-14, 1996 from four sites at the Station. Three samples were collected at Site 6; six samples at Site 7; ten samples at Site 9; and 32 samples at Site 19. The data consisted of composite soil samples collected at depths between 0 to 12 inches. The samples were analyzed for nitroaromatic/nitramine (explosives) analysis and/or particle size analysis. The sample locations and detected explosive compounds are presented on Figures 1 through 4.

The results of the soil characterization sampling effort were reviewed and discussed during the January 5, 1997 Treatability Study meeting conducted at LANTDIV's office in Norfolk, Virginia. Representatives from LANTDIV, WPNSTA Yorktown, WES, and Baker attended this meeting.

Based on the results, the group made the following conclusions: (1) the primary contaminants of concern appeared to be TNT, HMX, and RDX; (2) Site 9 did not appear to be a concern with respect to explosives contamination; (3) the composite TS soil sample would be collected from four areas at Sites 6, 7, and 19 which had the highest concentrations of explosives in the soil; and (4) the collection of the composite sample (100 gallons) would be based on a weighted average at each of the three sites: 70 percent collected at Site 19, 15 percent at Site 6, and 15 percent at Site 7.



100 0 50 100
1 inch = 100 ft.

BOUNDARY
DRAINAGE
MARSH
RAILROAD
FENCE
EDGE OF PAVEMENT
STRUCTURE
APPROXIMATE SITE DELINEATION

APPROXIMATE SAMPLE LOCATION (COMPOSITE)

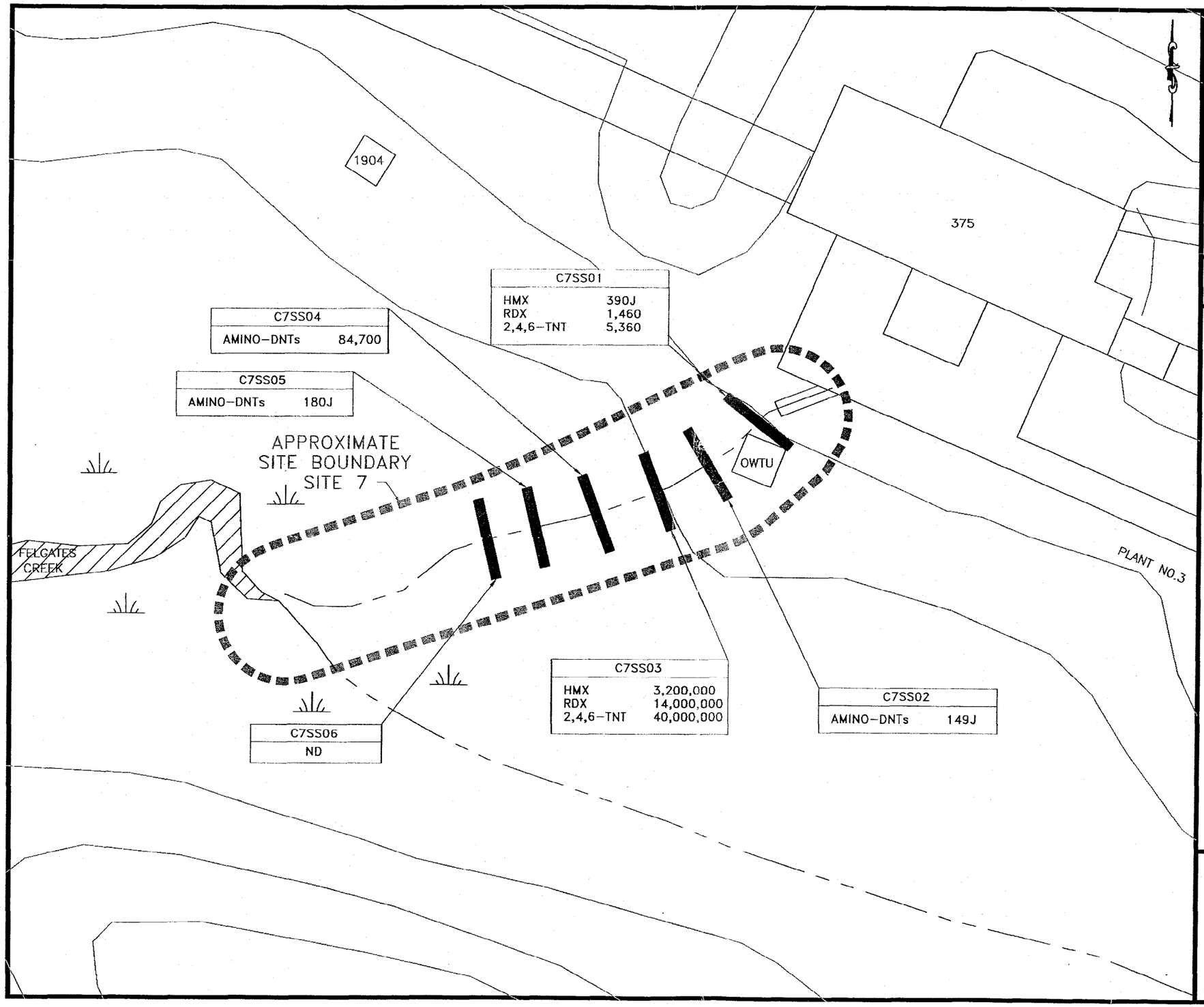
NOTES:
1. ALL CONCENTRATIONS ARE IN ug/kg UNITS.
2. ND = NOT DETECTED.

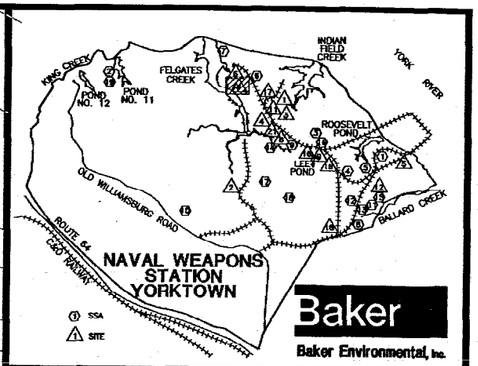
209504TS

FIGURE 1
PRELIMINARY SOIL CHARACTERIZATION RESULTS
SITE 6
EXPLOSIVES CONTAMINATED WASTEWATER IMPOUNDMENT

NAVAL WEAPONS STATION YORKTOWN YORKTOWN, VIRGINIA

01389 ABIZ





50 0 25 50

1 inch = 50 ft.

BOUNDARY
EDGE OF PAVEMENT
DRAINAGE
STRUCTURE
MARSH
APPROXIMATE SITE DELINEATION
RAILROAD
FENCE

APPROXIMATE SAMPLE LOCATION (COMPOSITE)

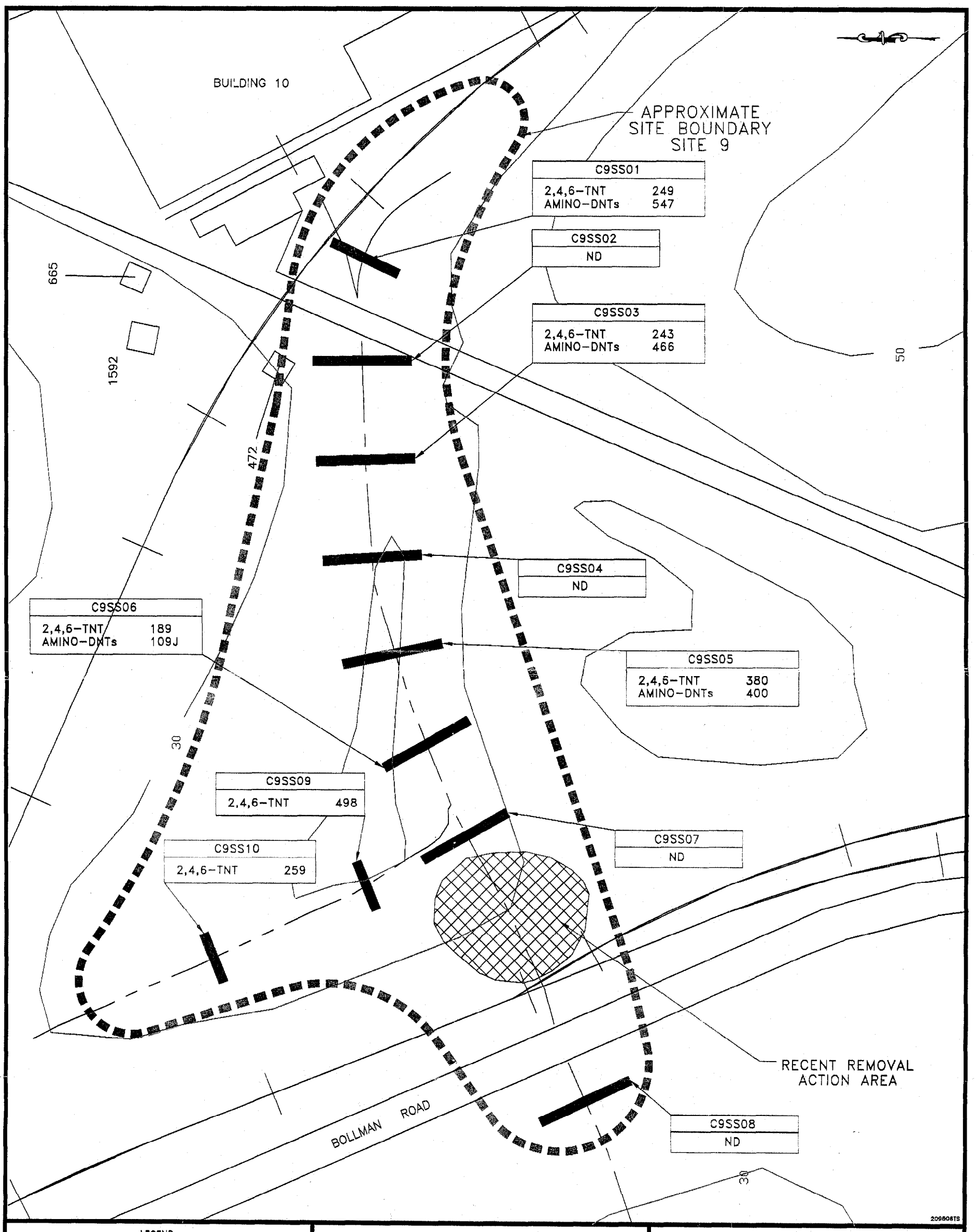
NOTES:

1. ALL CONCENTRATIONS ARE IN ug/kg UNITS.
2. ND = NOT DETECTED.

209505TS

FIGURE 2
PRELIMINARY SOIL CHARACTERIZATION RESULTS SITE 7 - PLANT 3 EXPLOSIVES - CONTAMINATED WASTEWATER DISCHARGE AREA

NAVAL WEAPONS STATION YORKTOWN YORKTOWN, VIRGINIA


BUILDING 10
APPROXIMATE SITE BOUNDARY SITE 9
C9SS01
2,4,6-TNT 249
AMINO-DNTs 547
C9SS02
ND
C9SS03
2,4,6-TNT 243
AMINO-DNTs 466
C9SS04
ND
C9SS05
2,4,6-TNT 380
AMINO-DNTs 400
C9SS06
2,4,6-TNT 189
AMINO-DNTs 109J
C9SS07
ND
C9SS08
ND
C9SS09
2,4,6-TNT 498
C9SS10
2,4,6-TNT 259
RECENT REMOVAL ACTION AREA
BOLLMAN ROAD
665
1592
472
30
50
30
LEGEND
BOUNDARY
DRAINAGE
MARSH
RAILROAD
FENCE
60 CONTOUR LINE
EDGE OF PAVEMENT
STRUCTURE
APPROXIMATE SITE DELINEATION
APPROXIMATE SAMPLE LOCATION (COMPOSITE)
NOTES:
1. ALL CONCENTRATIONS ARE IN ug/kg UNITS.
2. ND = NOT DETECTED.
KING CREEK
FELGATES CREEK
INDIAN FIELD CREEK
YORK RIVER
POND NO. 12
POND NO. 11
ROOSEVELT POND
LF#1 POND
OLD WILLIAMSBURG ROAD
ROUTE 64
BALLARD CREEK
NAVAL WEAPONS STATION YORKTOWN
SSA
SITE
Baker
Baker Environmental, Inc.
FIGURE 3
PRELIMINARY SOIL CHARACTERIZATION RESULTS
SITE 9 - PLANT 1 EXPLOSIVES CONTAMINATED WASTEWATER DISCHARGE AREA
NAVAL WEAPONS STATION YORKTOWN
YORKTOWN, VIRGINIA
50 0 25 50
1 inch = 50 ft.
209508TS

APPROXIMATE SITE BOUNDARY SITE 19

C19SS11
ND

C19SS12
ND

C19SS13
2,4,6-TNT 158

C19SS01
2,4,6-TNT 135
AMINO-DNTs 192

C19SS15
ND

C19SS14
2,4,6-TNT 1,250

C19SS17
ND

C19SS16
ND

C19SS02
2,4,6-TNT 69,600

C19SS03
2,4,6-TNT 398
2,4-DNT/2,6-DNT 279

C19SS19
2,4,6-TNT 1,460
AMINO-DNTs 1,010

C19SS18
2,4,6-TNT 278

C19SS04
ND

C19SS05
ND

C19SS07
2,4,-DNT/2,6-DNT 2,500

C19SS20
ND

C19SS06
ND

C19SS08
HMX 208
2,4,6-TNT 7,160

C19SS09
2,4,6-TNT 863,000

C19SS21
ND

C19SS10
2,4,6-TNT 5,280
AMINO-DNTs 5,650

C19SS28
2,4,6-TNT 8,000

C19SS22
ND

C19SS23
ND

C19SS24
ND

C19SS32
ND

C19SS30
ND

C19SS27
ND

C19SS31
ND

C19SS26
2,4,6-TNT 500

C19SS29
ND

C19SS25
ND

OVERHEAD CONVEYOR BELT

CONVEYOR BELT

BOLLMAN ROAD

Baker
Baker Environmental, Inc.

100 0 50 100
1 inch = 100 ft.

LEGEND

- BOUNDARY
- DRAINAGE
- MARSH
- RAILROAD
- FENCE
- 60 CONTOUR LINE
- EDGE OF PAVEMENT
- STRUCTURE
- APPROXIMATE SITE DELINEATION
- OR APPROXIMATE SAMPLE LOCATION (COMPOSITE)

NOTES:

1. ALL CONCENTRATIONS ARE IN ug/kg UNITS.
2. ND = NOT DETECTED.

209507TS

FIGURE 4
PRELIMINARY SOIL CHARACTERIZATION RESULTS
SITE 19
CONVEYOR BELT SOILS AT BUILDING 10

NAVAL WEAPONS STATION YORKTOWN YORKTOWN, VIRGINIA

3.2 Task I-2 - Collection, Homogenization, and Shipment of the Sample

Task I-2 (Collection, Homogenization, and Shipment of the Sample) was performed during the week of January 16, 1995. As previously mentioned, the composite TS sample was collected from Sites 6, 7, and 19. Approximately 35 to 40 gallons of soil were collected in the vicinity of sample location C19SS09 (refer to Figure 4) at Site 19. The soil was collected to a depth of 18 inches. The second sample area at Site 19 was near previous sample location C19SS02. Approximately 35 to 40 gallons of soil were collected around this sample point. Baker collected approximately 15 to 18 gallons of soil at Site 7 in the area between sample locations C7SS02 and C7SS03 and C7SS04 and C7SS05 (refer to Figure 2). At Site 6, approximately 15 to 18 gallons of soil were collected near the end of the concrete culverts and downstream of location C6SS02 (refer to Figure 1). The soil was wet and contained large amounts of clay. Baker homogenized the soil on site and then placed it into two 55-gallon steel drums.

Baker collected a representative soil sample from the composited soil and sent it to a laboratory for full Target Compound List (TCL) organic compound, Target Analyte List (TAL) inorganic compound, and nitroaromatic/nitramine analysis. The analytical results were used to determine the initial explosives concentrations and to determine what other contaminants may be present in the soil which could affect the TS.

Table 1 lists the compounds that were detected in this soil sample and their corresponding concentrations. As shown on the table, four explosives were detected in the soil sample: TNT, RDX, HMX, and 1,3,5-trinitrobenzene (1,3,5-TNB) at concentrations of 1,200,000 micrograms per kilogram (µg/kg); 190,000 µg/kg; 80,000 µg/kg; and 190 µg/kg, respectively. In addition, low levels of three volatile organic compounds (VOCs) and two pesticides were detected in the sample along with several inorganics.

The two 55-gallon drums of soil were sealed and shipped to the WES laboratory in Vicksburg, Mississippi in early February.

TABLE 1

SUMMARY OF COMPOUNDS DETECTED IN THE COMPOSITE TREATABILITY STUDY SAMPLE
NAVAL WEAPONS STATION YORKTOWN
YORKTOWN, VIRGINIA

Detected Compounds	Treatability Sample
Explosives: (µg/kg)	
HMX	80,000
RDX	190,000
1,3,5-Trinitrobenzene	190
TNT	1,200,000
Volatile Organic Compounds: (µg/kg)	
Acetone	29B
1,2-Dichloroethene	65
Trichloroethene	7J
Pesticides/PCBs: µg/kg)	
4,4'-DDE	6
4,4'-DDT	20
Total Inorganics: (mg/kg)	
Aluminum	8,410
Arsenic	7
Barium	52
Beryllium	1
Calcium	4,400
Cadmium	1
Cobalt	6
Chromium	21
Copper	21
Iron	22,100
Potassium	777
Magnesium	836
Manganese	150
Sodium	293
Nickel	15
Lead	47
Selenium	1
Vanadium	56
Zinc	142
Cyanide	1

Notes:

B = Compound was also detected in the laboratory blank.
J = Estimated value
µg/kg = micrograms per kilogram
mg/kg = milligrams per kilogram

3.3 Task I-3 - Soil Sample Storage

At the laboratory, the two 55-gallon drums of soil were stored in a padlocked refrigerator kept at 4°C. Small portions of soil (5 gallons) were removed from the drums when needed for individual experiments.

3.4 Task I-4 - Homogenize and Sieve Samples

Task I-4 (Homogenize and Sieve Samples) was initiated by WES once the two drums of soil were received and stored at the laboratory. At the WES laboratory, the soil sample was sieved with a sterilized USA Standard Testing Sieve No. 5. The wet soil was pushed through a mesh sieve. The sieved soil was placed into sterilized 5-gallon plastic buckets, as needed.

Since the soil had a high moisture and clay content, it was difficult to homogenize. The first attempts to homogenize the soil in the laboratory via a carboy and a hand-held mechanical mixer proved ineffective because the soil was dense and tended to clump together. Effective homogenization was obtained by mixing the soil by hand for 15 minutes. Therefore, the soil was homogenized by hand as needed.

3.5 Task I-5 - Chemical and Physical Characterization of the Soil Sample

Task I-5 (Chemical and Physical Characterization of the Soil Sample) was initiated by WES in February 1995 and completed in March. The physical characterization testing included sieve analysis and atterberg limits. The chemical characterization testing included: priority pollutants, explosives, cresols, hydrazines, heavy metals, pH, TOC, CEC, ammonia-nitrogen, nitrate/nitrite, and phosphates. During this task, WES determined that the wet soil introduced potential error into the analytical work. Therefore, a higher number of replicates had to be taken to reduce the error.

The results from the chemical characterization study (which included five replicates) are presented on Table 2. As shown on this table, the TNT concentrations ranged from 842 to 2,220 milligrams per kilogram (mg/kg).

TABLE 2

CHEMICAL CHARACTERIZATION RESULTS
NAVAL WEAPONS STATION YORKTOWN
YORKTOWN, VIRGINIA

Compounds	Replicate Sample Number				
	1	2	3	4	5
Explosives (mg/kg):					
TNT	1,090	2,220	900	880	842
2,A-Dinitrotoluene	102	64.5	47.5	64.5	71.5
4,A-Dinitrotoluene	63.5	33.5	25.5	38.5	40.5
2,6-Dinitrotoluene	<26.0	<26.0	<26.0	<26.0	<26.0
2,4-Dinitrotoluene	<25.0	<25.0	<25.0	<25.0	<25.0
HMX	150	138	87	114	112
RDX	415	436	250	325	336
Trinitrobenzene	<25.0	5.50J	<25.0	<25.0	<25.0
Dinitrobenzene	<25.0	<25.0	<25.0	<25.0	<25.0
Hydrazine	<0.025	<0.025	<0.025	<0.025	<0.025
Nutrients (mg/kg):					
TKN	1,144	671	799	847	1,263
N02-N	5.37	5.07	6.3	5.39	7.63
N03-N	37.9	32.8	51.8	40.3	38
NH3-N	27.8	15.2	16.4	21.2	16.7
TP	26.4	16.6	24.1	16.6	17.6
OP04	0.4	0.26	0.24	0.25	0.07
TOC	13,061	10,572	11,268	10,701	10,971
COD	6,234	5,634	5,993	15,334	6,988
pH	7.0	7.2	7.1	7.2	7.0
CEC	14.0	14.6	20.3	17.0	21.3

4.0 REMAINING TREATABILITY STUDY PHASES

Phases II through VII of the TS were completed entirely by WES and not Baker. Therefore, a discussion of the activities and the results will not be presented within the body of this report. Instead, a copy of WES's three-part report discussing the entire TS has been presented in Appendices A, B, and C. At this time, WES's report is in draft form. Final reports will be included once available.

APPENDIX A
A MICROBIOLOGICAL INVESTIGATION OF TRINITROTOLUENE-CONTAMINATED SOIL FROM YORKTOWN NAVAL WEAPONS STATION (DRAFT)

NOTE: **The attached report is the author's review draft. Per the author's request, the report should not be cited. A final report will be included once available.**

Technical Report
July 1996

US Army Corps of Engineers
Waterways Experiment Station

A MICROBIOLOGICAL INVESTIGATION OF TRINITROTOLUENE-CONTAMINATED SOIL FROM YORKTOWN NAVAL WEAPONS STATION

By *Wayne E. Evans*
AScI Corp.

Dr. Herbert Fredrickson, Dr. Doug Gunnison
EPED/EL

Major Steve Harvey
USA, EED/EL

Dr. Mark Zappi
Mississippi State University
Mississippi State, MS

Mr. Rich Stryker/ Mrs. Sherry Eng
Naval Facilities Engineering Command, Code 18222
Norfolk, VA

Mr. Jeffrey Harlow
Naval Weapons Station Yorktown
Yorktown, VA

Prepared for Headquarters, U.S. Army Corps of Engineers

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Strategic Environmental Research Development Program

Technical Report
July 1996

A MICROBIOLOGICAL INVESTIGATION OF TRINITROTOLUENE-CONTAMINATED SOIL FROM YORKTOWN NAVAL WEAPONS STATION

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Report 1 of a series

Approved for public release: distribution is unlimited

Prepared for U.S. Army Corps of Engineers
Washington DC 20314-1000

Under EIRP Work Unit 31627

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WES Diagram

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Preface

The work reported herein was conducted by the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, as part of remediation technology assessments by the Naval Facilities Engineering Command (NFEC), Atlantic Division 1824, Norfolk, Virginia. Additionally, this work was also part of the Strategic Environmental Research and Development Program (SERDP), Installation Restoration Research Program (IRRP) and the U.S. Army Environmental Quality Technology Research Program.

Personnel who cooperated in the execution of the study and the preparation of this report include Dr. Herbert Fredrickson, Principle Investigator, and Dr. Douglas Gunnison, Ecosystem and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL; Major Steve Harvey; Project Manager; Dr. Mark Zappi, Environmental Restoration Branch (ERB), Environmental Engineering Division(EED); Mr. Wayne E. Evans, Mr. Glenn Myrick, Mr. Todd French, Mr. Mike Ochman, Ms. Margaret Richmond, AScI Corporation, McLean, VA.

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This report should be cited as follows:

> Evans, W.E., Harvey, S. D., Fredrickson, H., Gunnison, D., Zappi, M.E. (1996). "A Microbiological Investigation of Trinitrotoluene-Contaminated Soil From Yorktown Naval Weapons Station" Technical Report, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

1 Introduction

Contaminants comprised of aromatic rings are most readily mineralized by aerobic bacteria which have the enzymatic systems required to oxidize aromatic rings and use molecular oxygen as their terminal electron acceptor (e.g. strains of the genus Pseudomonadales). However, mineralization of TNT under aerobic conditions can be greatly impeded by the formation of dead end metabolites and conjugation of TNT and its metabolites to organic matter in soils. These conjugates are thought to result from the formation of a covalent bond between the nitrogen-containing substituent on the explosive molecule and humic material in soils. Many of these products are more toxic than TNT itself. Reports have indicated TNT can be effectively mineralized under anaerobic conditions and the formation of these unwanted products can be minimized. In the anaerobic process the nitro-substituents of TNT are sequentially reduced to yield triaminotoluene which is subsequently oxidized to Kreb's cycle intermediates. Formation of conjugates with humic material appears to be minimized under anaerobic conditions.

Recent publications have reported a novel pathway for the mineralization of TNT. An aerobic pseudomonas was derived which sequentially removes the nitro-substituents from the aromatic ring yielding toluene. Toluene can be readily degraded via a number of aerobic (e.g. tol) and anerobic pathways. These pathways are both encoded on plasmids which have been mated into facultatively anaerobic bacteria. It should be possible to evaluate the nitro-removal pathway in both aerobic and anaerobic biotreatment systems.

It is not possible to know in advance whether an aerobic or an anaerobic biotreatment process will be the most effective for the remediation of Yorktown soils. Therefore, our initial screening of treatments included aerobic and anaerobic approaches.

Representative Yorktown soil samples were studied in microcosms designed to simulate bench scale bioslurry and biocell reactors. Bioslurry and biocell reactors are above ground reactors in which soil is placed and may be amended with cometabolites, nutrients, and microbial consortia. Bioslurries are continuously mixed whereas biocells are intermittantly mixed. Radiolabeled TNT was mixed into soil samples in the microcosms along with other amendments and incubated for 14 days.

The degradation of TNT was assayed by monitoring the disappearance of ^{14}C-TNT, the appearance of ^{14}C-labeled metabolites and the evolution of $^{14}CO_2$. The kinetics of evolution of $^{14}CO_2$ were determined by regular sampling of an aqueous KOH trap in the microcosm and analysis by liquid scintillation counter. The disappearance of ^{14}C-labeled TNT and the appearance of ^{14}C-labeled metabolites was determined at the end of the incubation period by extraction and analysis by silica gel thin layer chromatography (TLC) and autoradiography.

After incubation, treated soils will be extracted using the Bligh-Dyer extraction

method. Contaminants, natural products (lipids), and the extract were separated into 3 polarity classes by sequentially eluting them from silica gel chromatography column with dichloromethane, acetone, and methanol. The dichloromethane eluate was analyzed by TLC-autoradiography as described above. The polar membrane lipids in the methanol eluate was analyzed to provide information on the total microbial biomass in the soil.

The objectives of this study were to:

1. To assess and maximize the explosive-degrading potential of native Yorktown soil microbial communities using microcosms simulating bioslurry and biocell reactors.

2. To assess the efficacy of adding foreign explosive-degrading microorganisms to Yorktown soil.

3. To develop the experimental parameters to use for bench scale bioslurry (5 liter) and biocell (30 liter) reactors.

To accomplish the objectives listed above, the study was organized around the following tasks:

Task 1. Determine the most effective means to stimulate native microbes to degrade TNT by comparing rates of degradation in soils receiving amendments (Table 1) to the sterile control.

Task 2. Determine efficacy of adding exogenus organisms by comparison TNT degradation rates in treatments receiving microbial amendments to those that do not.

Task 3. Evaluate the effect of adding the surfactant Tween 80 to Yorktown soil during biotreatment. Previous studies at WES have shown that Tween 80 increased the mineralization of TNT in explosive contaminated soil. Comparison of the rates of TNT mineralization of soils receiving Tween 80 to their corresponding sterile control will determine its effectiveness.

Thus, the purpose of this research was to evaluate the degradation of both ^{14}C-TNT and TNT already present in the soil matrix ('cold' TNT) under various redox conditions (aerobic, microaerophilic, and anaerobic) with four cometabolites in flasks simulating biocell and bioslurry reactors. Additionally, the effect of amending soils with surfactant and the efficacy of bioaugmentation was assessed (Table 1). A mass balance of ^{14}C provided information on the efficacy of the treatments. This research was used to screen and identify conditions and cometabolites for use in bench scale treatability studies with biocell (30 liter) and bioslurry (5 liter) reactors. Explosives contaminated soil was obtained from the US Navy's Yorktown Naval Weapons Station, located at Yorktown, Virginia.

Table 1. Conditions, flask types, and treatments examined during study

Condition	Flask Type	Treatments						
Aerobic	Biocell	Hot Moisture Oxidation	Hot Moisture Oxidation with Tween 80	Tween 80	No Additives	Molasses	Toluene	Corn Syrup
Aerobic Anaerobic Micro-aerophilic	Bioslurry Biocell and Bioslurry Biocell and Bioslurry	Hot Moisture Oxidation	Hot Moisture Oxidation with Tween 80	Tween 80	No Additives	Molasses	Toluene	Potato Starch
Aerobic and Anaerobic	Bioaug-mented Biocell and Bioslurry	Hot Moisture Oxidation	Simplot Method	Simplot with Tween 80	Joliet Slurry	Joliet Slurry w/ Tween		

2 Materials and Methods

Collection and Treatment of Yorktown Soil

The Yorktown Naval Weapons Station Soil was collected from three TNT contaminated areas (sites 6, 7, and 9) at Yorktown Naval Weapons Station (YNWS), Virginia. Due to the varying concentrations of TNT contaminated soil, the soil was homogenized on the site to achieve a 1000 mg/kg (ppm) TNT concentration. The soil was placed into two 55-gallon steel drums. These drums were sealed and shipped to Waterways Experiment Station (WES). Upon arrival, the Yorktown soil was wet and contained large amounts of clay and centimeter size crystals of TNT. The two 55-gallon plastic buckets containing the Yorktown soil were placed in a padlocked 4°C refrigerator.

Soil Homogenization

The Yorktown soil was sieved with a sterilized USA Standard Testing Sieve No. 5, 4.0 mm (.157 in) Tyler Equivalent 5 Mesh (Fisher Scientific Company). The wet soil was pushed through the mesh sieve using a sterilized pestle and large spatulas. The sieved soil was placed into two 5- gallon plastic buckets tested for sterility. Sterility of the two 5-gallon collection buckets was determined by pouring sterile nutrient broth into the proposed collection bucket. After gently rotating the bucket, the nutrient broth was poured back into the original test tube. Overnight incubation and lack of turbidity in the tubes determined that the plastic buckets were sterile.

The first attempt to homogenization the soil via a carboy and a hand held mechanical mixer proved ineffective due to the clumping and density of the wet soil. The only method to homogenize the sieved soil was mixing by hand for 15 minutes. This homogenized soil was trandferred back into the two 5-gallon plastic buckets

Before any soil was used for experiments, the soil was mixed with a sterile spatula for two minutes to remove any micro-gradients that may have developed during storage.

Storage

The two 5-gallon buckets containing the sieved and homogenized YNWS soil were maintained in the padlocked 4°C refrigerator. When required, only enough soil was collected from each bucket for that particular experiment or study. Only one bucket at a time was removed from the cold room and replaced when that specific amount was obtained.

Sterile controls

Before the analysis of each portion of the biotreatability study, 400 g of YNWS soil was used to make sterile controls. The soil was aseptically weighed and transferred to a 500-ml Kimax glass beaker using an ethanol flamed spatula. The Kimax glass beaker was covered with aluminum foil. The beaker was autoclaved at 121°C for 30 minutes, allowed to sit overnight at room temperature, and autoclaved again. The doubled-autoclaved (also known as hot moisture oxidation) soil was used for the sterile soil controls with and without the addition of the surfactant Tween 80.

Before the initiation of each portion of the biotreatability study, a 0.1 ml aliquot of 30% slurry (autoclaved soil plus Staniers' Mineral Salts Media (MSM) with or without amendments and surfactants) was tested for sterility by plating onto a nutrient agar plate. Plates were inverted and incubated at a temperature of 30°C overnight. Appearance of colonies suggested preparation problems with autoclaved soil or Staniers' MSM. All solutions used were also checked for sterility via plating onto nutrient agar.

Physical and chemical characterization of soil

The physical and chemical characteristics of the soil were determined by analysis for explosive concentration, moisture content, pH, and total organic carbon content (Table 3and 4).

Oven dry weight

Five replicates, each containing 10-gram of soil, were weighed onto preweighed aluminum pans. Soil samples were dried for 24 hours in a 100°C oven. After cooling in a desiccator for 15 minutes, moisture content was determined by the difference in weight between the initial and final soil weight.

Soil particle size distribution

The soil particle size distribution was determined using a settling-out procedure. A 20-g wet soil sample (based on the moisture content) was added to 1 L of Reverse Osmosis (RO) water. This solution was shaken and allowed to settle overnight. The type of soil (silt, clay, sand, or combination of the three), the diameter and number of the particles, and the surface area were determined via the difference of the timed observations of the settling particles.

Total organic carbon

Using aseptic techniques, five replicates, each containing 0.250 g of oven dried pulverized soil was weighed into 24 hr combusted ceramic crucibles. The oven dried soil was pulverized using a combusted ceramic pestle and mortar. The weighed soil and the

standards (each for inorganic and total organic carbon) were analyzed using the Shimadzu TOC-5000/5050 Solid Sample Analyzer. The organic carbon content was determined from the difference between the total organic carbon and the inorganic carbon.

Soil pH

The pH (or the hydrogen/-log[H+] concentration) of the soil was determined using a combination electrode attached to an Orion pH meter.

Chemical Analysis of Soil

The table below lists the analysis peformed on the soil and its referenced procedure.

Table 2. Analysis performed for chemical characterization of soil

Anaylsis	Reference
TKN	EPA Method 351.2
NO2-N	EPA Method 353.2
NO3-N	EPA Method 353.2
NH3-N	EPA Method 350.1
TP	EPA Method 365.4
OP04	EPA Method 365.1
TOC	Standard Methods 5310 D
COD	EPA Method 410.4
pH	EPA Method 410.4
CEC	[1]EPA/CE-81-1 p3-20

[1]Procedures for Handling and Chemical Analysis for Water and Soils

TNT concentration (HPLC-DAD

Using aseptic techniques, five replicates, each containing 5.0 g of Yorktown soil were extracted using the Bligh-Dyer (B-D) extraction method. The extraction of the soil samples determined the initial explosives content (TNT, monoamino, diamino, azoxy compounds, RDX and HMX). TNT and its transformation products were separated by High Performance Liquid Chromatography (Hewlett-Packard 1090) on reversed phase C18 columns (flow rate 1.5 ml/min; mobile phase-68% of a 20-mM ammonium chloride solution and 32% of a 98% methanol-2% butanol mixture) and detected with a diode array detector (Jenkins et. al., 1994).

Microbiological Characterization of Soil

The microbial biomass of the soil, the structure of the microbial community, and the potential of the native community to metabolize natural ^{14}C-labeled organic substances comprised the microbiological characteristics of the soil.

Direct (dapi-microscopic) bacterial count

The native biomass of the Yorktown soil microbial community was assayed by using a Fluorescent Direct Count (FDC) method. Using aseptic techniques, 2.5 g of soil was stained with the fluorescent dye Acridine Orange-DAPI. The observed number of fluorescing bacteria enumerated per gram of soil was considered the total number of the cells/gram of soil.

Indirect (serial dilution) bacterial plate counts

The aerobic heterotrophic biomass of the Yorktown soil microbial community was determined using a serial dilution plate count. A 10 g soil sample was diluted in 90 ml of sterile Mineral Salts Broth (MSB) and serially diluted (using the milk dilution procedure) to achieve 10^{-2} to 10^{-9} range. A 0.1 ml aliquot of slurry was plated by onto a nutrient agar plate. The plates were inverted and incubated at a temperature of 30°C overnight.

Catabolic potential

The metabolic activity of the native Yorktown soil microbial community was assayed by challenging soil samples with ^{14}C-labeled acetate and ^{14}C-labeled glucose in separate respirometer flasks. The rate of $^{14}CO_2$ evolution and the rate of incorporation of ^{14}C into microbial polar lipids provided information on the catabolic potential (or health) of the soil microflora. This two-day incubation study was done under various oxidation-reduction conditions (aerobic and anaerobic) and moisture contents (30% bioslurry and biocell). The procedure used to determine the catabolic potential was essentially the same procedure as that used for the biotreatability study.

Biotreatability studies

Biotreatability studies were done on the TNT contaminated Yorktown soil to evaluate the explosive-degrading potential of the native microflora under various conditions. Additionally, the native microflora were bioaugmentated with known TNT degraders were also added to the native microflora under various environmental conditions to determine examine explosive-degrading potential under conditions of bioaugmentation.

Biocell and Bioslurry Flasks

Two-hundred and fifty ml Bellco Biometer flasks were incubated at various electron acceptor or oxygen tension conditions of aerobic (weakly reducing at +25 mV), microaerophilic (mildly reducing at 0 mV +/-20 mV), and anaerobic (-100 mV)). Twenty grams of Yorktown soil was placed into each flask before the addition of any treatments to simulate the Biocell treatment. Approximately 70 ml of a 30% (w/v) slurry was placed into additional flasks before the addition of any treatments to simulate the Bioslurry treatment.

Microcosms

All treatments were pre-made in stock solutions with Staniers' MSM and stored in 4 C. All treatments were performed in replicates of seven and are as follows:

(1) carbon sources of Molasses, Potato Starch, or Toluene at concentrations of 1%, 1%, or 20 milliMolar, respectively.

(2) the surfactant Tween 80 at a concentration of 1% (w/dry soil wt).

(3) sterile control of Tween 80 at 1% (w/v).

(4) a no additive sterile control (autoclaved) consisting of nutrients (nitrogen and phosphorus) but no carbon source.

(5) a no additive consisting of nutrients but no carbon source.

Additionally, the benefits of bioaugmentation were assessed. The treatment conditions were as follows:

(1) the added TNT degrading microbes.

(2) the added TNT degrading microbes with 1% Tween 80 surfactant (w/v).

(3) a no additive sterile control.

The anaerobic bioaugmentation portion of the biotreatability study consisted of a addition of TNT degrading consortia from Joliet Army Munitions Depot/Argonne National Labs (Joliet Slurry) and Simplot (Crawford and Crawford from University of Idaho University/Simplot Method). All anaerobic work was done in the Coy Anaerobic Chambers. The aerobic bioaugmentation portion of the biotreatability study consisted of TNT degrading consortia from WES (Hastings Triplets isolated from Hastings Army Ammunitions Depot Sediment).

A 10-ml aliquot of Joliet slurry was added to 20 g of Yorktown soil in simulated TNT contaminated biocells and bioslurries. Molasses (0.3%) was added to each of these simulations. Tween 80 (1% w/v) was added to half the cells and slurries. Oxygen reduction potential (ORP) and pH was analyzed and maintained at every analysis or sample period. The ORP was maintained at anoxic conditions (-200 mV).

The Simplot Method (Crawford and Crawford from University of Idaho University) consisted of Potato starch, Phosphate Buffer Saline (PBS) solution, and freeze dried consortia. The Potato starch and freeze dried consortia were stored in the 4°C

refrigerator. A calculated amount of freeze dried consortia and potato starch was added to 20 g of Yorktown soil in contaminated TNT simulated biocells and bioslurries. The surfactant Tween 80 (1% v/v) was added to half the cells and slurries. Oxygen reduction potential (ORP) and pH was analyzed and maintained at every analysis or sample period. The ORP was maintained at anaerobic conditions (-100 mV).

The Hastings Triplet was inoculated into 100 ml of 100 ppm TNT Staniers' MSM 5 days before the initial aerobic bioaugmentation date. The solution was centrifuged at 7,000 RPM's (6,895 x g) using a Sorvall Ultra Centrifuge for 30 minutes. The pellet (containing the consortia) was washed three times with Staniers' MSM. The final rinse concentrated the consortia into 50 ml. Aerobically, a 20-ml aliquot of the concentrated Hastings Triplet of Hastings microbes was inoculated to 20 g of Yorktown soil in TNT contaminated biocell and bioslurry simulations. The surfactant Tween 80 was added to half the slurries and cells.

Electron Acceptor Conditions

Aerobic soil slurry and biocell simulations were incubated at a temperature of 30°C and in an aerobic environment having an Eh of 50 mV. Bioslurry simulations were incubated on a rotary shaker at 100 rpm's, while biocell simulations were incubated on a stationary shelf. All rubber stoppers were greased to prevent loss of $^{14}CO_2$, thus insuring collection of radiolabeled $^{14}CO_2$ products.

Microaerophilic soil slurry and biocell simulations were performed according to aerobic conditions in an anoxic (microaerophilic) environments having an Eh of 0 mV. The anoxic environment was obtained by purging the biometer flask with argon gas for two minutes at the beginning of the study, at each collection period, and after fresh base was added to the side arm flasks. The biometer flasks were incubated in New Brunswick Psychrotherm incubators under an atmosphere consisting of ultra pure nitrogen. The pH, ORP, and dissolved oxygen were monitored on sample collection days on all replicates. All rubber stoppers, buret tops, and candelabras were glued with epoxy to the biometer flask to limit oxygen trandfer, to prevent loss of radiolabeled gas, and to insure the collection of radiolabeled gas.

The anaerobic soil slurry and biocell simulations were incubated in the Coy and Plaslab anaerobic chambers filled with a nitrogen and hydrogen (96% N_2/4% H_2) gas mixture. Bioslurry simulations were incubated on a rotary shaker at 100 rpm while biocell simulations were incubated on a stationary shelf. The chambers were purged with ultra pure nitrogen before the injection of the nitrogen/hydrogen mixture. The chamber was maintained at a minimum 4% H_2 and 0% O_2 gas level. The anaerobic chambers were allowed to equilibrate for one day before any initial analysis was collected. All anaerobic portions were done at oxidation reduction potential of -200 mV. The pH and ORP were monitored on sample collection days on all replicates. All radiolabeled mineralization collection sampling was performed inside the anaerobic chamber. On the last day of incubation, the chamber was allowed to become aerobic.

Radiochemical Analysis

A total of 0.9 uCi (200,000 DPM'S) of u-ring-[C^{14}]-TNT was added to each of the slurry and biocell replicates. The radiolabeled compound was added in diluted sterile aqueous solution to insure a homogenous mixture. Radiolabeled CO_2 was collected in 1 N titrated potassium hydroxide (KOH).

The KOH carbon dioxide traps were changed on days 1, 2, 3, 5, 7, 10, 14 of the experiment. The samples were harvested on day 14 and analyzed radiochemically. A mass balance of ^{14}C and its distribution among the phases (carbon dioxide, aqueous supernatant, and soil pellet) was determined. Soil and aqueous samples were extracted. The ^{14}C products were characterized by thin layer chromatography (TLC) and autoradiography. The non-radiolabeled products were characterized by HPLC-DAD and TLC.

A Bligh-Dyer (B-D) extraction was performed on the soils, while a salting out extraction was conducted on each of the aqueous samples. The B-D extraction served as the first analytical step for determining the biomass and community structure (via lipid analysis) of the soil. The B-D extraction was also performed to prepare the soil samples for determination of explosive compounds (TNT, monoamino-, diamino-, etc.). After extraction, further analysis consisted of HPLC-DAD and TLC. Based on the analytical results a mass balance was determined.

The Bligh-Dyer extraction method was chosen due to its ability to provide accurate measurements TNT concentration in Weldon Springs soil (a soil used in a past biotreatability study). A comparison of the B-D method with other solvents is provided in Table 3.

In the first phase of the B-D extraction process (Figure1), the soil sample was extracted with methanol-dichloromethane-water (MeOH-DCM-H_2O) in the ratio 2:1:0.8, respectively. This single phase solvent system was miscible with soil pore water, was a good wetting agent, and rapidly melts polar lipids in cell membranes. The extraction mixture was vortexed for one minute and treated in an ultrasonic bath for one hour to ensure efficient extraction. After setting for 18 hours the liquid phases of the extraction mixture were separated by the addition of DCM and water to produce a final MeOH-DCM-H_2O ratio of 2:2:0.9. Solid materials were separated by centrifugation at 12,000 RPM (17,369 x g) for 30 minutes using a Sorvell Centrifuge. Non-polar contaminants and natural products (lipids, total lipids) were recovered in the DCM phase.

The total solvent extractable material from the soil in the recovered DCM phase was fractionated into three polarity classes by sequential elution through a column packed with silica gel (SiO_2). The first solvent (DCM) eluted TNT and most of its transformation products. Acetone and methanol, the second and third solvent respectively, eluted the polar microbial membrane lipids used to determine microbial biomass and community

structure. All solvents were concentrated using the Organomation Associates, Inc. N-EVAP and stored at -20 °C until further analysis or dilutions were conducted.

Table 3. Comparison of varous TNT extracton methods with various solvents on Weldon Springs Soil.

Solvent Extraction Method	TNT (ug/g)	
	Mean	STD Dev
Acetonitrile (Air dried soil)	956.1	40.3
Acetonitrile (Wet soil)	844.4	79.4
Bligh-Dyer DCM (Air dried soil)	1025.5	66.7
Bligh-Dyer DCM (Wet soil)	899.9	18.1
Bligh-Dyer MeOH (Air dried soil)	50.2	6.8
Bligh-Dyer MeOH (Wet soil)	45.4	3.6
1-Butanol (Air dried soil)	1014.4	73.2
1-Butanol (Wet soil)	968.2	74.7
Dimethyl Sulfoxide (Air dried soil)	590.8	54.2
Dimethyl Sulfoxide (Wet soil)	818.9	119.1
Ethanol (Air dried soil)	996.9	80.6
Ethanol (Wet soil)	805.3	96.9
Methanol (Air dried soil)	997.8	60.6
Methanol (Wet soil)	1027.3	337.5
1-Propanol (Air dried soil)	910.3	94.8
1-Propanol (Wet soil)	505.0	48.9
2-propanol (Air dried soil)	822.1	159.7
2-Propanol (Wet soil)	856.7	227.6

AIR DRIED SOIL = Air dried soils extracted with 10 ml of solvent and sonicated overnight
WET SOIL = Air dried soil (~1g) plus 1 ml water and sonicated for 8 hr
9ml of solvent added and sonicated for 8 hr.

A salting-out procedure was done on all supernatant (aqueous) samples collected before the first phase of B-D extraction. This was accomplished by over-saturating a 5-ml aliquot of an aqueous sample with sodium chloride (NaCl) plus 2 ml of acetonitrile (ACN). The saturated salt solution caused the TNT and its transformation products to partition into the ACN phase. This salted-out phase was concentrated on Organomation Associates, Inc. N-EVAP. A second rinse was used on the concentrated salted-out phase

and transferred to another DCM washed tubes to avoid interference of salt crystals in the HPLC column. This salted-out phase was again concentrated on the Organomation Associates, Inc. N-EVAP, and the samples were stored at -20 C. The HPLC extraction used in the soil preparation was performed on the aqueous samples.

$^{14}CO_2$ (Carbon Dioxide) Quantitation

The endogenous rate of mineralization was determined via measurements of the absorbed radiolabeled carbon dioxide in the standardized base (1N KOH) over a two-week incubation period. A mass balance was determined from the analysis of the three various phases of the radioactive biotreatability study. Collection of these phases, quantitation of the radioactivity in each phase using a Packard Model 2500 Liquid Scintillation Spectrometer, and a computation of the percent radiolabeled material in these phases gave overall mass balance. A B-D extraction of the soil prepared the soil sample for the analysis of accumulation of TNT transformation products and the reduction of TNT via autoradiography from TLC.

At each collection time, one ml aliquot of each KOH samples containing adsorbed $^{14}CO_2$ was placed into a 20-ml liquid scintillation vial, each containing 15 ml of Ultima Gold™ cocktail solution. Fresh 1 N KOH was replaced into the empty well. The $^{14}CO_2$ was quantified on the Liquid Scintillation Spectrometer (LSC). The slurry was acidified with four drops of concentrated phosphoric acid and allowed to incubate an additional 24 hrs in the presence of 1 ml of fresh 1N KOH. At this time a final KOH sample was taken and the $^{14}CO_2$ (tied-up as bicarbonates) was determined. The rate of accumulation of $^{14}CO_2$ was used to decide the mineralization rate of the sample. The overall amount of radioactivity in the base also provided the first phase of the radioactive mass balance.

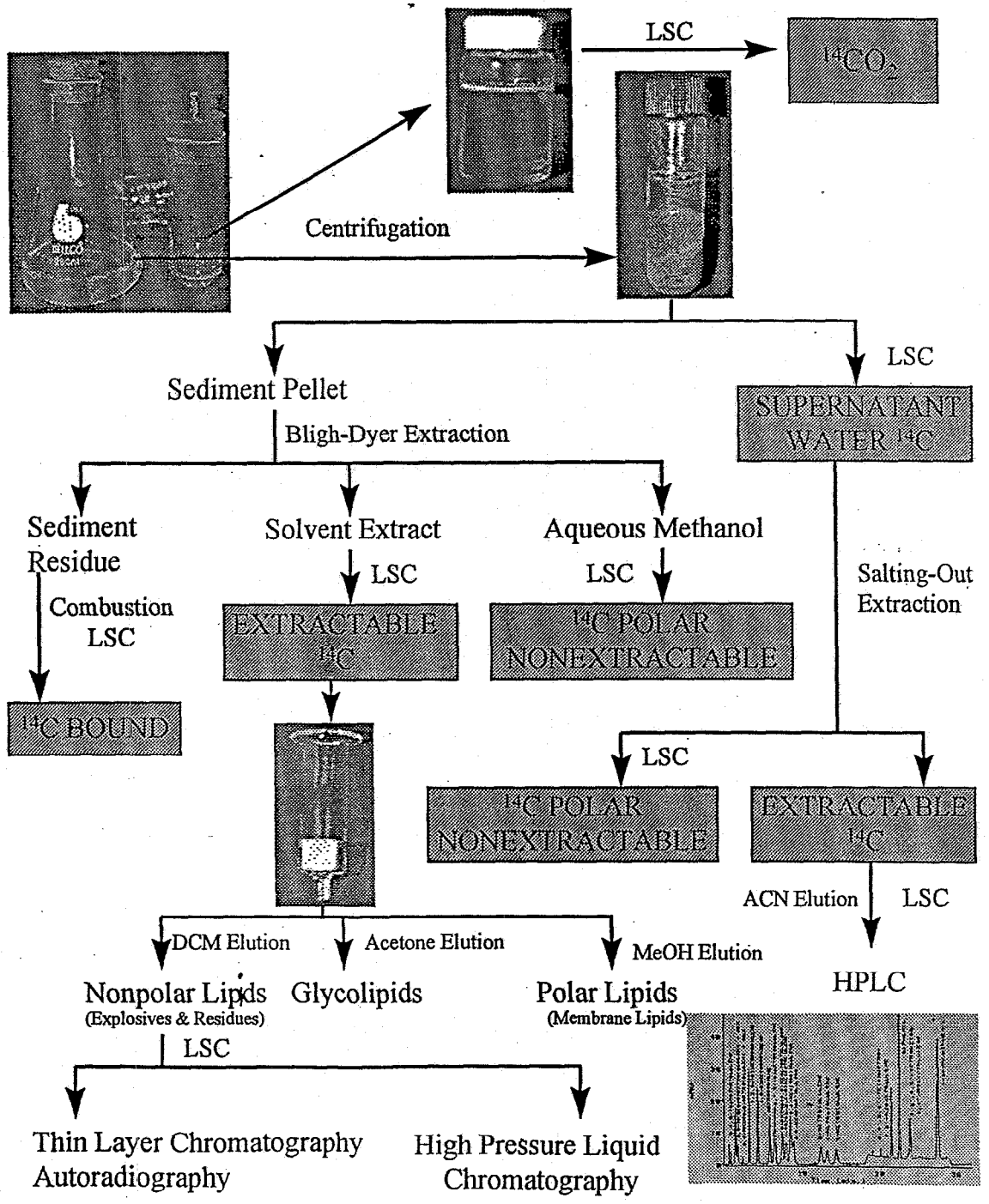
^{14}C in Supernatant

Radioactivity in the supernatant provided the second phase of the radioactive mass balance. Following collection, the slurry (from biocell and bioslurry simulations) was centrifuged (17,369 x g for 30 minutes) using the Sorvall SS-34 Centrifuge to separate the liquid and solid phases. A one ml aliquot of each supernatant sample was prepared and counted as for the $^{14}CO_2$ quantitation work. The salting out extraction procedure was performed on the remaining supernatant. The radiocarbon present in 0.1 ml aliquot of salted-out phase (acetonitrile) and the remaining supernatant (aqueous) was also determined using LSC. The overall radioactivity of the supernatant was a combination of the extracted (acetonitrile) salted-out phase and the non-extracted (aqueous) phase.

^{14}C in Soil

Radioactivity in the soil was determined by summation of the radioactivity present in the extracted (DCM), the non-extracted (MeOH/aqueous), and combustible (oxidizable) phases. Following collection and removal of the supernatant, a B-D extraction was performed on the remaining pellet. After centrifugation of the second

Sample Fractionation/Extraction Scheme



phase addition, both the DCM and MeOH/aqueous phases were collected separately into DCM-washed tubes. After the DCM phase was concentrated (previously discussed), the ^{14}C present in a 0.1 ml aliquot was determined by LSC. Once the MeOH/aqueous phase was collected, a 1 ml aliquot was placed into 15 ml of Ultima Gold cocktail and counted (as previously discussed).

Following collection of the DCM and MeOH phases, the extracted soil was prepared for oxidation analysis. Subsamples each containing 0.2 g of an extracted pellet were weighed into triplicate oxidizer cups and funnels. The extracted pellet was oxidized on a Packard Model 307 Solid Sample Oxidizer. Radiolabeled carbon dioxide released from the oxidized materials was collected in 15 ml of Ultima gold cocktail, The radiolabeled content was determined by LSC. In addition, an oven dry weight was determined on 1.0 g of the extracted pellet. This accumulation of radiolabeled products in the pellet comprised the third phase of the mass balance.

^{14}C Mass Balance

Results of the B-D extraction and the salting out procedure quantified the amount of ^{14}C in following three fractions:

(1) ^{14}C present as $^{14}CO_2$ (tied-up as bicarbonates) in the KOH well.
(2) ^{14}C in the soil was further defined as that radioactivity that was:
    a. extractable (DCM fraction)
    b. non-extractable (MeOH)
    c. combustible (oxidizable)
(3) ^{14}C present in the supernatant (aqueous phase of the slurry)

The analysis of the three different fractions (KOH, aqueous, and soil phases) determined the percentages of radiolabeled products and provided an overall mass balance. These radiolabeled products and their fraction were important in determining the total mass balance of each portion of the biotreatability study. The mass balance determined the appearance of the radiolabeled (transformation) products and the disappearance of the radiolabeled TNT.

Chemical analysis

Chemical analysis of bioslurries and biocells were performed after two-week incubation period to determine the status of TNT in both the radiolabeled (new) and non-radiolabeled (old) material.

High Performance Liquid Chromatograph (HPLC) with Diode Array Detector (DAD) analysis

TNT and its transformation products were separated by HPLC on a reverse phase C-18 column (flow rate 1.5 ml/min; mobile phase-68% of a 20 mM ammonium chloride

solution and 32% of a 98% methanol/2% butanol mixture) and detected with a DAD (Figure).

Soil and aqueous samples were prepared by adding 100 μL of methanol to the concentrated sample. A 50-μL aliquot of the concentrated sample extract was transferredinto 2.95 ml of a 50/50 methanol/Milli-Q water mixture. A 25-μL aliquot of this extract was injected onto the HPLC.

Thin layer chromatography (TLC)

The appearance of TNT transformation products and the disappearance of TNT were determined by analysis of the soil and supernatant with TLC to separate components in the concentrated DCM or acetonitrile extracts, respectively.

A 10 μL concentrated DCM or ACN sample was spotted onto a Carbosorb Fluorescent 450 TLC plate. Separation of the products in the DCM extract was accomplished by analysis of the plate in a solvent system containing 99.0% toluene and 1% methanol for approximately one hour. The R_f values for the radioactive TNT and TNT transformation products were determined by analysis with the Ambus Optical Imaging Microscope. Identities of the compounds were established by comparison with known standards.

Autoradiography

The appearance of radiolabeled products and the disappearance of radiolabeled TNT were determined by analysis of the soil and supernatant with TLC to separate components in the concentrated DCM or acetonitrile extracts, respectively.

The 10 μL concentrated DCM or ACN samples were spotted onto a Carbosorb Fluorescent 450 TLC plate. The procedure used in the analysis of non-radiolabeled products for TLC was used for the radioactive products. Radioactive standards were used in the place of non-radioactive products.

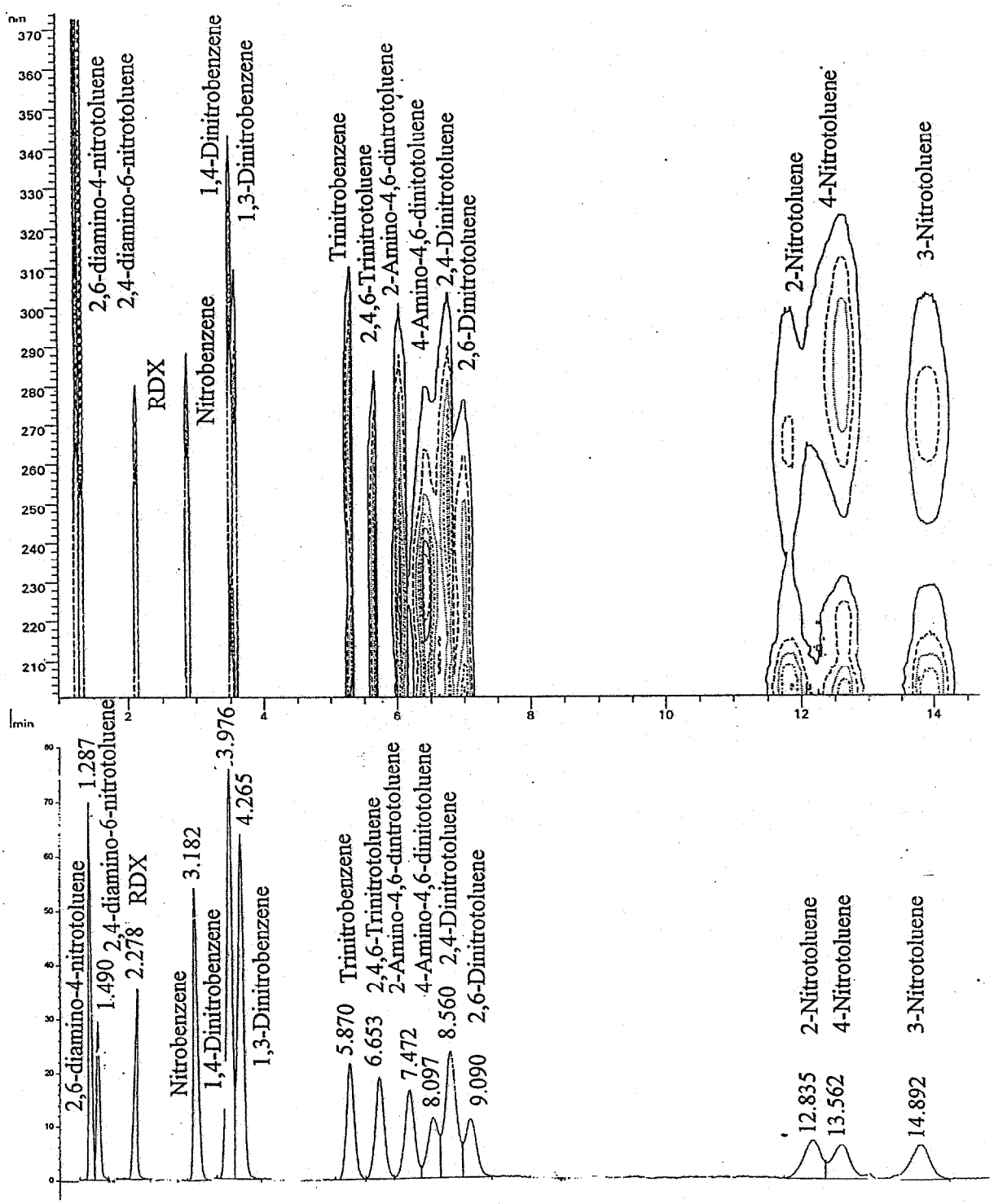


Figure 2. HPLC Chromatograph of Explosives Compounds

RESULTS

The results of the physical characterization of the soil are provided in Table 4 and the chemical characterization is provided in Table 5.

Table 4. Physical characterization of soil

	Treatments	Values
1)	Oven Dry Weights	
	% Solids	75.66%
	% Moisture	24.34%
2)	pH	6.56
3)	Soil Particle Distribution	
	% Clay	10%
	% Silt	10%
	% Sand	80%
4)	Total Organic Carbon	
	% Organic Carbon	2.40%
	% Inorganic Carbon	0.01%
5)	Biomass	
	Direct Count	$2.7x10^5$ Cells/g dw
	Indirect Count	$1.7x10^5$ Cells/g
6)	Initial Explosive Concentration	
	TNT	644 ppm
	RDX	400 ppm
	2-Amino-4,6-Dinitrotoluene	86 ppm
	4-Amino-2,6-Dinitrotoluene	96 ppm

Table 5. Chemical characterization of soil

Sample #	TKN	NO2-N	NO3-N	NH3-N	TP	OP04	TOC	COD	pH	CEC
1	1144	5.37	37.9	27.8	26.4	0.4	13061	6234	7.0	14
2	671	5.07	32.8	15.2	16.6	0.26	10572	5634	7.2	14.6
3	799	6.3	51.8	16.4	24.1	0.24	11268	5993	7.1	20.3
4	847	5.39	40.3	21.2	16.6	0.25	10701	15334	7.2	17
5	1263	7.63	38	16.7	17.6	0.07	10971	6988	7.0	21.3

Analysis of Radiolabeled TNT

$^{14}CO_2$ production

The first portion of the overall mass balance consisted of radioactivity absorbed into the standardized 1N KOH. The amount of $^{14}CO_2$ absorbed would determine the endogenous rate of mineralization of the uniformly ring lableled ^{14}C-TNT.

When radiolabeled glucose or acetate were used as cometabolites for the Yorktown TNT contaminated soil, the cumulative $^{14}CO_2$ production over a 30-hr incubation period amounted to approximately 27.4% and 35.9% for glucose (aerobic and anaerobic) and 40.3% for acetate, respectively (Figure 3). This high activity observed with the Yorktown Soil indicates that the native consortia were viable and active for these radiolabeled consumables. Under anaerobic conditions, with a glucose addition, a closure of mass balance was not achieved indicating the possible production of $^{14}CH_4$ or other volatile compound that could not be trapped by the KOH.

The production of $^{14}CO_2$ from the radiolabeled TNT incubated in both biocell and bioslurry reactors (under aerobic, microaerophilic, anaerobic and bioaugmentation conditions) were generally less than 2% (Figures 4 -13). The total $^{14}CO_2$ released was well below the 3 % minimum needed to account for the 97% purity of the radiolabeled TNT prepared. Thus, the radiolabeled TNT was neither consumed nor transferred directly into radiolabeled carbon dioxide by the native consortia or the bioaugmented treatments.

^{14}C in soil

The radioactivity in the soil was determined for the second fraction of the overall mass balance. The total amount of radioactivity was determined from the sum found in the extractable (ie radioactivity contained in DCM), the non-extractable (radioactivity contained in MeOH), and the combustible soil fraction (radioactivity that could not be extracted from the soil). As expected, most of the radioactivity was found in the soil fraction.

TNT and its transformation products are detected in the extractable (DCM) phase. A small amount of radioactivity (i.e. ^{14}C-TNT) was found in the DCM phase. The radiolabeled products extracted in this phase were TNT and two TNT metabolites (2A-4,6 DNT and 4A-2,6 DNT). Other TNT transformation products were not detected or found to be negligible.

Radioactivity incorporated into the cell membrane was detected in the non-extractable (MeOH) phase. The results showed very little amounts of radioactivity in the non-extractable phase. This suggested that very few native Yorktown consortia were actively incorporating ^{14}C-TNT into its cell mass. The inability of the consortia to incorporate ^{14}C-TNT into the cell membrane was also shown for the bioaugmentation portion of the experiment. Thus, both native and added TNT degrading microbes were unable to show ^{14}C-TNT uptake.

After Bligh-Dyer extractions were performed on the pellet, the soil sample was combusted to determined the amount of radiolabeled products remaining bound to the soil. Most of the radiolabeled carbon was detected in the combusted phase for both bioslurry and biocell reactors under aerobic, microaerophilic, and bioaugmentation conditions (Figures 4-13). The combusted phase consisted of bound ^{14}C-TNT, its transformation products, and cell membranes.

Under microaerophilic, anaerobic native and anaerobic bioaugmentation bioslurry studies (Figures 5,6,8,10, and 11) the mass balance is not closed. Additionally, the amount of radioactivity detected in the soil combustible phase in the anaerobic bioslurry is only half the concentration as compared to the aerobic and microaerophilic combustible portions. This 'missing mass balance' and the decrease in the combustible soil concentration was noticed in the anaerobic catabolic potential using radiolabeled glucose.

^{14}C in aqueous phase

Radioactivity in the supernatant was the third and final fraction of the overall mass balance. The radioactive aqueous phase was also extracted and separated into an extractable and non-extractable portion.

Very little of the radioactivity added initially was detected in the aqueous phase of the catabolic potential study. A salting-out extraction was not performed on the supernatant because of the high levels of radioactivity formed in the carbon dioxide and sediment.

In the biocells, the amount of radioactivity extracted in the sediment phase was approximately equal to the amount extracted in the aqueous phase. This concentration was was much lower than the radioactivity in the bioslurries and is probably a due to the small amount of water added to the biocells - approximately 4 milliliters (ml).

In the bioslurries, the amount of radioactivity extracted in the sediment phase was much lower than that found in the aqueous phase. This is due to the fact that the bioslurries had 70 mls of distilled deionized water added. Overall, the radioactivity detected in the aqueous phase was nearly half to one third of the radioactivity detected in the soil pellet of the 30% bioslurry.

Very low levels of radioactivity (radiolabeled explosives and transformation products) were detected in the ACN phase of the salting-out extraction performed on both the native microflora and bioaugmentation portion of the bioslurries (Figures 4,5,6, and 8). Most of the radioactivity was detected in the aqueous portion consisting of non-extractable products.

Missing ^{14}C

The largest portion of the mass balance of radioactivity was accounted for in the three phases, except for the anaerobic (both native and bioaugmentation) portions. In the anaerobic experiment the overall mass balances were low (approximately 50% and lower). As previously mentioned, the radioactivity detected in the combustible phase was nearly half the amount detected in the aerobic and microaerophilic combustible phases. Two plausible explanations for the missing ^{14}C is that it may have been uptaken by the microbial population or it may not have been adsorbed by the KOH trap (^{14}C in the form of short chained fatty acids).

Summary of Radiolabeled Results

Based on the initial study using radiolabeled glucose and acetate, the soil appeared to have a viable microbial population as a high percentages of CO_2 was produced. However, none of the treatments (aerated, microaerophilic, anaerobic, and bioaugmentation) nor any of the reactors (biocell or bioslurr) resulted in the mineralization of ^{14}C-TNT as the $^{14}CO_2$ produced did not exceed the impurities in the radiolabled TNT. The majority of the ^{14}C was found to be bound to the soil fraction. As a result of the thin layer chromatography analysis, transformation products of TNT were identified indicating the reduction of TNT. The very low amounts of ^{14}C in either the soil DCM extract indicates that only very small quantities of the ^{14}C-TNT may have been incorporated into the cells. Finally, the low mass balance of ^{14}C under microaerophilic and anaerobic conditions may be due to the production of volatile compounds.

Analysis of non-radiolabeled TNT

In addition to determining the fate of the radioactive TNT, the non-radiolabeled TNT present in the soil was also analyzed. Thus, the treatments are identical to those discussed previously. The analyses of the non-radiolabeled TNT (Figures 14-22) and its transformation products were performed using the same extractions as were used in the radiolabeled analysis.

Aerobic Bioslurry and Biocell

As discussed, the Hot Moisture Oxidation treatments were intended to be sterile controls by two treatments in an autoclave. The results of double autoclaving appear to be effective in transforming TNT and RDX.

In the bioslurry flasks, Molasses, Toluene, and the Hot Moisture Oxidation treatment showed the highest concentration of 2A-4,6 DNT and 4A-2,6 DNT which would indicate reduction of TNT (Figure 14). Tween 80 and the Potato Starch treatments had lower concentrations of TNT, but the 2A-4,6 DNT and 4A-2,6 DNT concentrations did not increase as would be expected if TNT were biotransformed.

In the biocell flasks (Figure 15), the Tween 80 treatment showed a reduction in TNT concentration with an increase in 2A-4,6 DNT and 4A-2,6 DNT concentrations.

The No Additives, Molasses, and Corn Syrup treatments all show very small concentrations of TNT and amino-DNTs.

A comparison of the bioslurry and biocell data (Figures 14 and 15) indicates that biotransformation of TNT is occuring in the bioslurry reactors as those treatments have higher concentrations of transformation products. The biocells generally have a much lower final concentration of TNT in the treatments

Microaerophilic Bioslurry and Biocell

In the bioslurry flasks (Figure 16), the Tween 80, Molasses, Toluene, and Potato Starch treatments all showed a lower concentration of TNT. The Tween 80, Molasses and Potato Starch treatments all showed an increase in amino-DNT concentration as compared to the initial soil concentration.

In the biocell flasks (Figure 17), the Tween 80 and Toluene treatments showed the greatest formation of amino-DNTs. Molasses and Potato Starch had the lowest overall concentrations of TNT and amino-DNTs.

A comparison of bioslurry and biocell data (Figures 16 and 17) shows that the bioslurry flasks have the lower final concentration of explosive compounds. It appears that the bioslurry flasks may have resulted in a faster stepwise reduction of TNT and its transformation products as they are lower than the biocell concentrations.

Anaerobic Bioslurry and Biocell

In the bioslurry flasks (Figure 18), the Tween 80 showed the greatest formation of amino-DNTcompounds. The Molasses, Toluene, and Potato Starch treatments all showed much lower TNT and amino-DNT concentrations than the initial soil concentration.

In the biocell flasks (Figure 19),all treatments showed very low concentrations of TNT and amino-DNTs.

A comparison of bioslurry and biocell data (Figures 18 and 19) show the benefit of anaerobic conditions as all treatments result in very low final concentrations of TNT and amino-DNT compounds.

Aerobic Bioaugmented Bioslurry and Biocell

Figure 20 contains the results of both bioslurry and biocell treatments. Both bioaugmented treatments show very high formation of amino-DNT as compared to the initial soil sample. It appears that the amino-DNT concentrations are lower in the bioslurry flasks which may indicate a faster transformation than in the biocell. The bioaugmentation bioslurry and biocell did have higher final explosive concentrations than the aerobic, microaerophilic, and anaerobic systems.

Anaerobic Bioaugmentation Bioslurry and Biocell

In the bioslurry flasks (Figure 21), all treatments showed an increase in the TNT transformation products. The Simplot method with Tween 80 had the highest concentrations of amino-DNT compounds. The Joliet slurry and Joliet slurry with Tween 80 addition were remarkably similiar in concentration of explosive compounds.

In the biocell flasks (Figure 22), the Simplot method with Tween 80 showed the greatest formation of amino-DNT compounds. The Joliet slurry and Joliet slurry with Tween 80 showed the lowest overall explosives concentration.

A comparison of bioslurry and biocell data (Figures 21 and 22) indicates that the bioslurry flasks showed the highest concentration of transformation products (amino-DNTs) however, the biocell flasks had the lowest final concentration of TNT.

Summary of Non-Radiolabeled TNT Results

The two most important TNT metabolites detected were 4A-2,6 DNT and 2A-4,6 DNT at varying concentrations. The Molasses and Potato Starch treatments consistently showed low concentrations of TNT and amino-DNT compounds. The Tween 80 treatment also performed well under most conditions and showed the formation of amino-DNT compounds in the anaerobic bioslurry and microaerophilic biocell.

In the anaerobic bioaugmented flasks, the Simplot and Joliet Surry showed lower overall explosive concentrations in the biocell as compared to the bioslurry. Both aerobic and anaerobic bioaugmented flasks showed the formation of transformation products in concentrations greater than those found under aerobic, microaerophilic, and anaerobic conditions.

The bioslurry flasks generally showed the greater formation of transformation products as compared to the biocells. However, the bioaugmented flasks showed the greatest formation of transformation products as compared to aerobic, anaerobic, and bioaugmented conditions.

DISCUSSION

Initial concentration of explosive compounds

The initial concentration for TNT and its metabolites was determined when the soil was received from the Yorktown Naval Weapons Station. Based on the results from the comparison of extraction methods (Table 2), the Bligh-Dyer extraction was used for determining the initial concentration of explosives in the Yorktown soil. The initial concentration of the soil was approximately 640 mg TNT/gram soil (dry weight). This differed from the TNT concentrations obtained from the initial collection (at the Yorktown site) and the WES base analytical determination (approximately 1,200 mg TNT/g soil). However, these other extraction methods required that the soil be air-died and pulverized (Method 8330) prior to extraction. Analyzing compounds from dried soil does not allow quantification of radiolabeled carbon contained in the cell mass, its storage location (glycolipid), determination of microbial genus, and whether the explosives (both radiolabeled and non-radiolabeled) were extractable or non-extractable from soil. Therefore, the Bligh-Dyer extraction technique was chosen as it provided more detailed information as to the fate of TNT.

This TNT concentration obtained via Bligh-Dyer extraction was the initial amount of TNT that served for the comparison of all the biotreatability treatments. A soil sample was not collected at the beginning of every biotreatability treatment.

The mass balances for the radiolabeled portion of the studies were based on the approximately 0.09 μCi (200,000 DPM's) of radiolabeled material (glucose, acetate, or TNT) added at the beginning of each biotreatability study. A determination of the amount of radiolabeled TNT present in solution at the beginning on each biotreatability treatment was made for comparison of possible radiolabeled TNT degradation. Based on the results of the purity check, the amount of radiolabeled TNT in that solution was 97%. Thus, radiolabeled carbon dioxide must be in the excess of 3.0% to indicate a possible or potential success in TNT mineralization to carbon dioxide.

Sterile Controls

The sterile controls consisted of soil that was twice autoclaved and had ^{14}TNT added. The sterile controls showed no significant production of $^{14}CO_2$. The amount of non-radiolabeled TNT was drastically reduced in both sterile control (one with no additives and one with Tween 80) as compared to the initial level of TNT. An increase of the monoamino-dinitrotoluenes and other transformation products was observed. The appearance of these transformation metabolites in the sterile controls helped to verify that the TNT was reduced.

Recent experiments have determined that the double autoclaving reduces the TNT into common transformation products such as 2A-4,6 DNT and 4A-2,6 DNT (unpublished results-Harvey, Evans, Fredrickson, Zappi). Based on these results, the sterile controls obtained by double-autoclaving should be renamed to 'Hot Moisture Oxidation

Treatments'.

Due to the results of double-autoclaving the soil, a true sterile control was not used in this study. During the biotreatability study, other forms of sterility were performed instead of double-autoclaving the soil samples. The addition of mercuric chloride has been a common practice in microbiology to sterilize of soil and inhibit microbial activity. However, recent experiments have proved that this form of sterilization of the soil also has some chemical disadvantages. The addition of mercuric chloride and clay reduces the TNT into common transformation products such as 2A-4,6 DNT and 4A-2,6 DNT (unpublished results-Harvey, Larson, Evans, Fredrickson, Zappi).

All sterile controls were checked for contamination and sterility effectiveness. Aerobic sterile controls incubated on nutrient agar plates had microbial growth. However, this growth was attributed to the Stanier's MSM and not the YNWS double-autoclaved soil. During preparation of the Stanier's MSM broth, a key ingredient was not properly sterilized which contaminated the Stanier's MSM Broth. However,the microbial contamination was not contributing to the mineralization of TNT. This was verified by the low rate of mineralization of the radiolabeled TNT into radiolabled carbon dioxide. Also, the TNT reduction was mostly attributed to the chemical processes of hot moisture oxidation and mercuric chloride addition rather than microbial reactions.

Effects of oxygen

The catabolic potential experiment (addition of radiolabeled acetate and glucose) to the YNWS sediment indicated that the sediment was healthy under both aerobic an anaerobic conditions (Figure). However, low levels of carbon dioxide were produced and recovered in all of the various oxidation-reduction regimes tested (aerobic, microaerophilic, and anaerobic).

The anaerobic mass balances were well below 100% of the added radiolabeled tracer for the studies utilizing natural microflora, bioaugmentation, and catabolic potential. Although low levels of carbon dioxide were produced, the amount or percentage of TNT (both non-radiolabeled and radiolabeled) had drastically decreased. In addition the quantitative level of radiolabeled products in the supernatant and soil pellet were approximately the same. The radioactivity detected in the anaerobic solid phase was nearly half the amount detected in the combustible phases under aerobic and microaerophilic conditions. Both soil pellet and supernatant phases had low amounts of radiolabeled TNT, amino-nitrotoluene (A-DNT), and diamino-nitrotoluene (DA-NT). This was also verified by the analysis with HPLC-DAD of low amounts of non-radiolabeled TNT and its transformation products. The low level of radiolabeled carbon dioxide combined with a low amount of radioactive products in the soil pellet was an indication that other volatile compounds were produced. The low amount of non-radiolabeled TNT and its transformation detected via HPLC-DAD analysis also verified that volatile compounds such as methane or short chain fatty acids (>5 Carbons) were likely to have been produced. Due to the experimental design these other volatile compounds were not detected or analyzed.

An acetonitrile rinse was performed on the biometer flasks to account for any loss of radioactivity due to sorption to glassware. After being washed, all radiolabeled glassware was rinsed with acetone and counted on the LSC. The results of both procedures suggested that the amounts of radioactivity in these rinses were negligible.

Other explanations for a low mass balance could be due to respirometer flask construction and to the acidification process. One concern was that the respirometer flasks were not properly sealed during the incubation period, allowing the radiolabeled carbon dioxide to be lost. However, replacing most of the biometer flask with a different, more tight fitting biometer flask has proven that leakage from the old biometer flask was not a major problem. Another related concern was that not enough phosphoric acid was added to end the biological reaction in the respirometer flask. However, after the studies were completed, samples of the acidify slurries were randomly tested using the pH meter and all samples registered a pH of 2.0.

The mass balances of the aerobic, microaerophilic, and aerobic bioaugmentation were approximately the same with some minor exceptions. The low amounts of radiolabeled carbon dioxide produced were associated with the fact that two thirds of the radioactivity was detected in the pellet (namely the combusted portion). TNT and its transformation products of 4A-2,6 DNT and 2A-4,6 DNT were found in the pellet phase, while no or very few radioactive products were detected in the aqueous phase.

Microaerophilic biotreatability was ineffective as determined by the high TNT concentration and low TNT transformation products in both slurries and cells. With the microaerophilic biotreatability study, the major concern was the maintaining an low oxygen (anoxic) environment.

Effect of added carbon/energy sources

Since the cumulative level of radiolabeled carbon dioxide formed in each of the treatments of added carbon sources (potato starch, molasses, and toluene) was below the 3 percent minimum needed to account for the 97% purity of the TNT solution it is not possible to differentiate on the optimal carbon source.

The overall quantitation of TNT and its transformation products (both radiolabeled and non-radiolabeled) of the no additives treatment indicated that this treatment does not readily degrade TNT. Further studies using a no additive treatment focusing on the monitoring the concentration of nitrogen (N) and potassium (P) was performed. TNT and RDX concentrations were equivalent to the initial concentrations. This no additive treatment with N and P monitoring was a better control than the no additive treatment that was used in the biotreatability treatments.

The TNT levels of molasses and potato starch treatments were much lower when compared with the initial levels of TNT and its transformation products. It appears that

these carbon sources provided an environment that enhanced Eh adaptation of the native microflora for interaction with TNT. By adding enough carbon to the medium it is possible that the environment shifted to a lower oxygen reduction potential, enabling the native microbes to have a higher affinity for the TNT molecule.

The addition of toluene as a carbon or energy source suggested that biological destruction of TNT may not have been very vigorous. In all treatments using toluene as a carbon source, a 10 fold reduction of TNT and increase of transformation products very rarely occurred. This is verified by the high percentage of radiolabeled products (mainly TNT) in the sediment extraction phase of DCM in which explosives were identified. This could suggest that the toluene oxygenases may not play an important factor in degrading TNT or that killed the microbes.

Effect of added surfactant

The amount of cumulative radiolabeled carbon dioxide released from the surfactant-amended treatments was below the 3% required for the studies.

The TNT concentration appeared to increase with the addition of the surfactant Tween 80. In many treatments there were very few differences in the disappearance of TNT and the appearance of TNT transformation products. Past experiments showed that surfactants can increase the availability of TNT to the microbes, thus desorbing the TNT from the soil (Zappi et al. in publication).

The amount of radioactivity in the combustible portion of the sediment phase was generally lower than that in other amendments with carbon sources. As compared to the biocells, the bioslurries appeared to have a lower percentage of radioactivity . This could be attributed to the greater availability of the TNT in the slurry then the biocell.

Bioaugmentation

The cumulative amount of carbon dioxide produced in the anaerobic bioaugmentation biotreatability study using the Simplot Method or the Joliet slurry was below the amount determined by the purity check. This was unusual, since both anaerobic methods have been shown to degrade TNT, with cumulative production of high levels of carbon dioxide. However, the low mineralization rate may be misleading with respect to reactivity of the Simplot Method and the Joliet Slurry. This difference could be attributed to the time span of the experiment. The 14 day study may not have been of sufficient for the exogenous organisms to adapt. The short time span of the experiment probably contributed to the small production of $^{14}CO_2$.

Quantitation of TNT and its transformation products following the 14-day incubation period indicted that both the Joliet Slurry and Simplot Method (30% bioslurries and biocells) showed a considerable decrease in TNT with reference to the initial concentration. Mono-aminodinitrotoluenes and other transformation products were detected using the Simplot Method included 2,6-DNT, 1,6-DNB, and the nitrotoluenes (2- and 4- NT). Use of surfactants with the Simplot Method produced no transformation

products of TNT. The TNT concentration varied little from the intial assessment. The surfactant (as previously mentioned) could have desorbed the TNT from the sediment which increased the amount available to the native and augmented microbes, yet little was mineralized.

The Joliet Slurry Biocells demonstrated a decrease in TNT in comparison to the 30% bioslurries. Monoaminodinitrotoluenes, other transformation products such as 2,4 DNT, and some unknown transformation products were detected on the HPLC-DAD. Thus, the Joliet Slurry had some degradation activity toward TNT. The addition of the surfactant Tween 80 also enhanced the disappearance of TNT.

The anaerobic bioaugmentation also displayed the same missing radiolabeled carbon in its mass balance as discussed previously. This confirms that some other volatile gas or short chain fatty acid is being produced and not detected due to the collection and analytical procedures employed.

Although both Simplot Method and Joliet Slurry were successful, analysis of radioactivity in the methanol phase after Bligh-Dyer extraction suggested differently. The radioactivity incorporated into the cell membrane was very low. This suggests that very few native microflora were actively incorporating TNT into its cell mass. The bioaugmentation portion confirmed this pattern with the native and amended TNT degrading microbes.

Although the cumulative amount of carbon dioxide was lower than the required 3%, the Hastings triplet of the aerobic bioaugmentation biotreatability study displayed potential activity as compared with the anaerobic bioaugmentation portions. The Hastings rate of mineralization was the best in any of the bioaugmentation portions. However, both slurry and cell simulations suggested that the very little non-radiolabeled TNT was being trandformed into potential transformation materials. The addition of the surfactant Tween 80 did not benefit the rate of mineralization or enhance the transformation of TNT in either slurry or cell.

Biocell vs. Bioslurry

The moisture content is important in the degradation of TNT. The bioslurries (30%) appear to be more effective in the removal of TNT. The mass balances were tighter, the rates of mineralization were much higher, and the non-radiolabeled TNT disappearances were better when compared with the biocells. This could suggest that the very nature of the slurry was as important. More TNT was available and released in the solution from the sediment due to the rapid rotation.

Due to the low moisture content of the biocells and the absorption of the little moisture available, many biocells did not have supernatant for analysis. Bligh-Dyer extraction of biocell for the sterile controls and no amendments were very dry. To maintain a low moisture content for the biocell very little moisture was added. The only

moisture in the biocell simulations were the moisture originally detected in the sediment, the 2 ml of solution added for carbon source or surfactant, and the 2 ml of radiolabeled TNT. Thus, the supernatant was not available for analysis for radioactive percentage. However, it would be expected that the radioactive and non-radioactive TNT would bind to the soil and that the high percentage of radioactivity would be detected in the soil pellet (combustible materials) phase.

As expected the biocells had less percentage of radioactivity in the aqueous phase as compared to the bioslurries. Analysis of the radioactivity detected in the aqueous phase was half that detected in the soil. Further analysis of the supernatant demonstrated very low amounts of radioactivity in the salting-out extraction performed on both the native microflora and bioaugmentation portion. Very little radioactivity (radiolabeled explosives and transformation products) were detected in the acetonitrile/salted-out extraction. Most of the radioactivity was detected in the aqueous portion consisting of non-extractable products. These non-extractable products were those products that were bound to the radiolabeled TNT and its transformation products such as lignens, plant products, and small suspended soil particles.

RDX

Although no radiolabeled RDX was added to the slurries and biocells, non-radiolabeled RDX was detected in the preliminary initial explosive analysis by HPLC-DAD. In addition to analyzing for TNT and its transformation products, RDX and possible transformation products were analyzed. RDX differs form TNT in that it has a triazine ring which makes it much more difficult to degrade.

As with TNT, the hot moisture oxidation and mercuric chloride addition both had shown a drastic reduction of RDX as compared to the intial concentration. These results suggest that RDX is reactive with the chemical properties involved with hot moisture oxidation and mercuric chloride addition. Very few transformation products were detected.

The non-radiolabled data indicates that RDX was disappearing in both aerobic and anaerobic conditions as compared of the initial concentration. Overall quantitation of RDX of the unamended (no additives) treatment indicated that this treatment does not readily degrade RDX. The RDX levels of molasses and potato starch treatments were much lower as compared with the intial amounts of RDX. It appears that the amount these carbon sources provided an environment that was quite adaptable for the native microflora to enhance it interaction with the RDX. By adding enough carbon to the media it is possible that the environment was shifted to a lower oxygen reduction potential allowing for a higher affinity of the native microbe reaction to the RDX molecule. The addition of toluene as a carbon or energy also suggested that it may help in the disappearance of RDX.

The addition of the surfactant Tween 80 did appear to enhance the disappearance of RDX. The concentration of RDX in the sediment after Bligh-Dyer extraction was

much lower as compared to the initial RDX concentration. There was no increase of concentration of RDX as detected in the desorption of TNT when Tween 80 was added.

The addition of known TNT degraders did not enhance the removal of RDX from the sediment. This was expected since the anaerobic microbes added were isolated from TNT contaminated sites with very little or no RDX detected.

There was no difference between biocell and bioslurry treatments in the reduction or disappearance of RDX.

Conclusions

General

There was no indication of any direct mineralization of TNT to carbon dioxide. However, the disappearance of TNT and the formation of some transformation products in some treatments were observed. Based on the study results, both molasses and potato starch treatments should be further investigated under aerobic and anaerobic conditions

Most of the TNT (radiolabeled and non-radiolabeled) was present in the non-extractable solids phase. It appeared that the TNT bound to the soil particles, plant remnants, or amendments particles (i.e., potato starch and molasses particulate).

Surfactants appeared to enhance the degradation of TNT and RDX. It was possible that the surfactant Tween 80 released the explosives and made it more readily available for the microbial or chemical reaction to occur. Further studies using various surfactants and concentrations would be beneficial and should be conducted to determine the effects of the surfactants on the native and added microflora. It may be possible that the 1% surfactant concentration was the optimal concentration.

All anaerobic work (native microflora and bioaugmentation) displayed a low or 'missing' mass balance. Based on our results where low carbon dioxide production and low radioactivity in the combustible phase of the pellet were observed, it seems likely that volatile gases or fatty acids were produced. Derivatives of the products should be performed on all anaerobic work to determine the fatty acids produced. In addition, other forms of monitoring the evolution of volatile gases should be determined.

Bioaugmentation demonstrated some of the best potential for TNT and RDX degradation based on by the disappearance of the explosive, the appearance of explosive transformation products, and the emergence of unknown products. Further bioaugmentation work should incorporate the Simplot Method.

Selection of Candidate Treatment Options

Bench scale studies incorporating biocell (30 liter) and bioslurry (5 liter) reactors follow this work. Table 5 details the candidate treatments, reactors, and conditions recommended for the bench scale research. In some cases, treatments are replicated in both biocell and bioslurry reactors in order to differentiate the benefit of mixing.

The sterile controls consist of mercuric chloride addition in order to sterilize the soil. The purpose of the sterile control is to determine the significance of other treatments and and abiotic processes.

Table 5. Treatment Conditions for Bioslurry and Biocell Reactors			
	Bioslurry		**Biocell**
Reactor #	**Aerobic**	Reactor #	**Aerobic**
1	Sterile Control	1	Sterile Control
2,3	No additives	2,3	Tw 80 & Molasses
4,5	Tw 80 & Molasses		**Anaerobic**
	Anaerobic	4,5	Potato Starch
6,7	Potato Starch	6,7	Tw 80 & Molasses
8,9	Simplot	8,9	Simplot
10,11	Simplot w/ 4hrs mixing	10,11	Molasses
12	Sterile Control	12	Sterile Control

The Tween 80 and Molasses treatment is a combination of the two treatments. Both Tween 80 and Molasses conditions showed mixed results in the shake flask study. Tween 80 showed good degradation in the bioslurry but not in the biocell. Molasses showed good degradation in the biocell but not in the bioslurry. Molasses has also proved to be a good cometabolite in remediation activities at Joliet Army Ammunition Plant, Illinois. The use of Tween 80 reduced the soil residence time by half in prior research. It is anticipated that Tween 80 will make the explosives more available and the molasses will stimulate the microbes to rapidly reduce the explosive compounds.

Potato Starch is the cometabolite for the Simplot process. Potato Starch showed good results in the anaerobic study and is a relatively available and cheap carbon source. The use of Potato Starch will allow comparison between the addition of exogenuous organisms (Simplot) and native consortia.

The Joliet process showed promising results, however, the process would require the shipment of Joliet microorganisms which would be unrealistic at the large scale. Thus, Molasses was chosen as a substrate due to its success at Joliet AAP and it is also cheap and readily available.

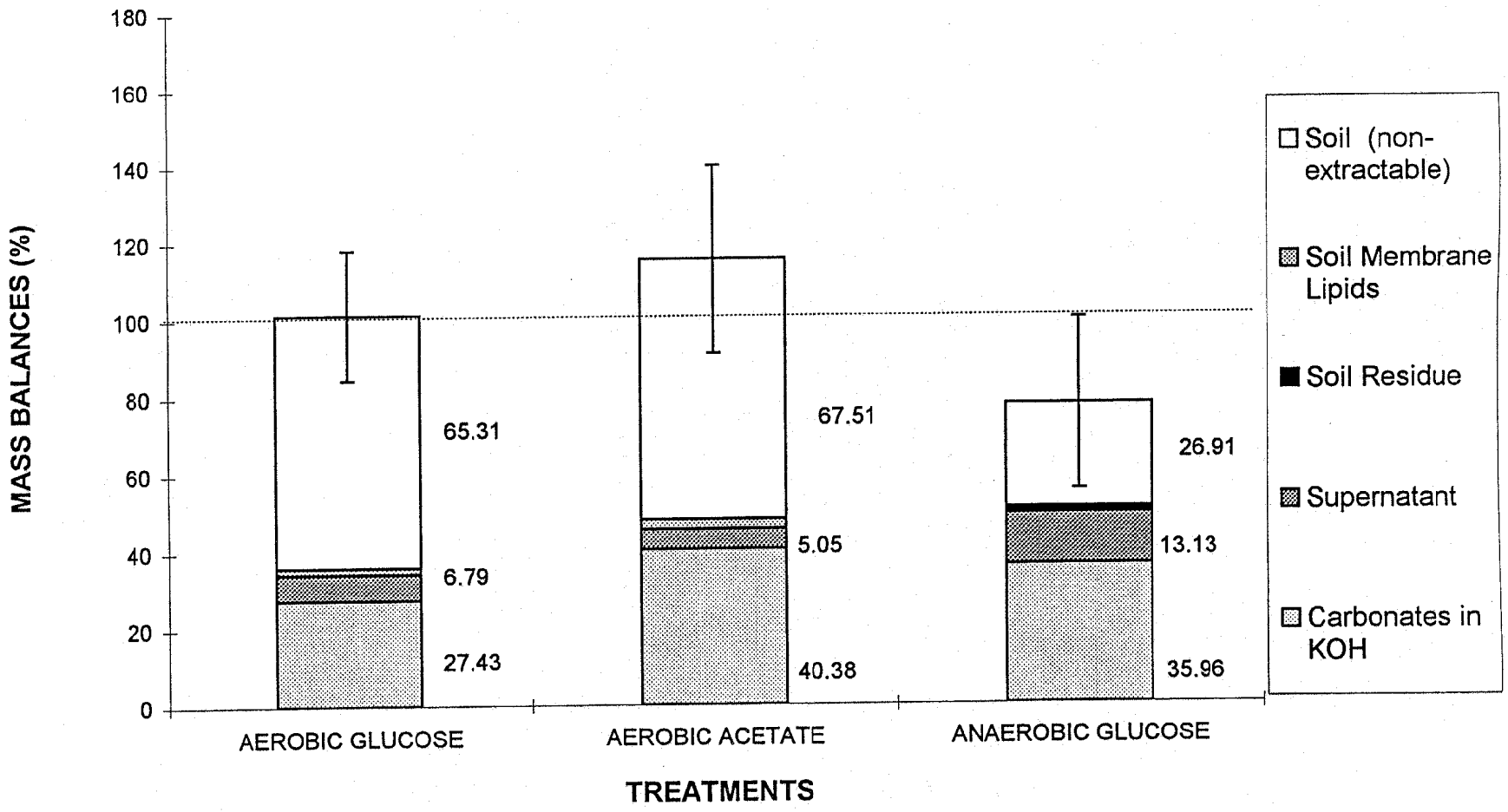


Figure 3. Mass balance of radiolabeled glucose and acetate with native consortia under anaerobic and aerobic conditions

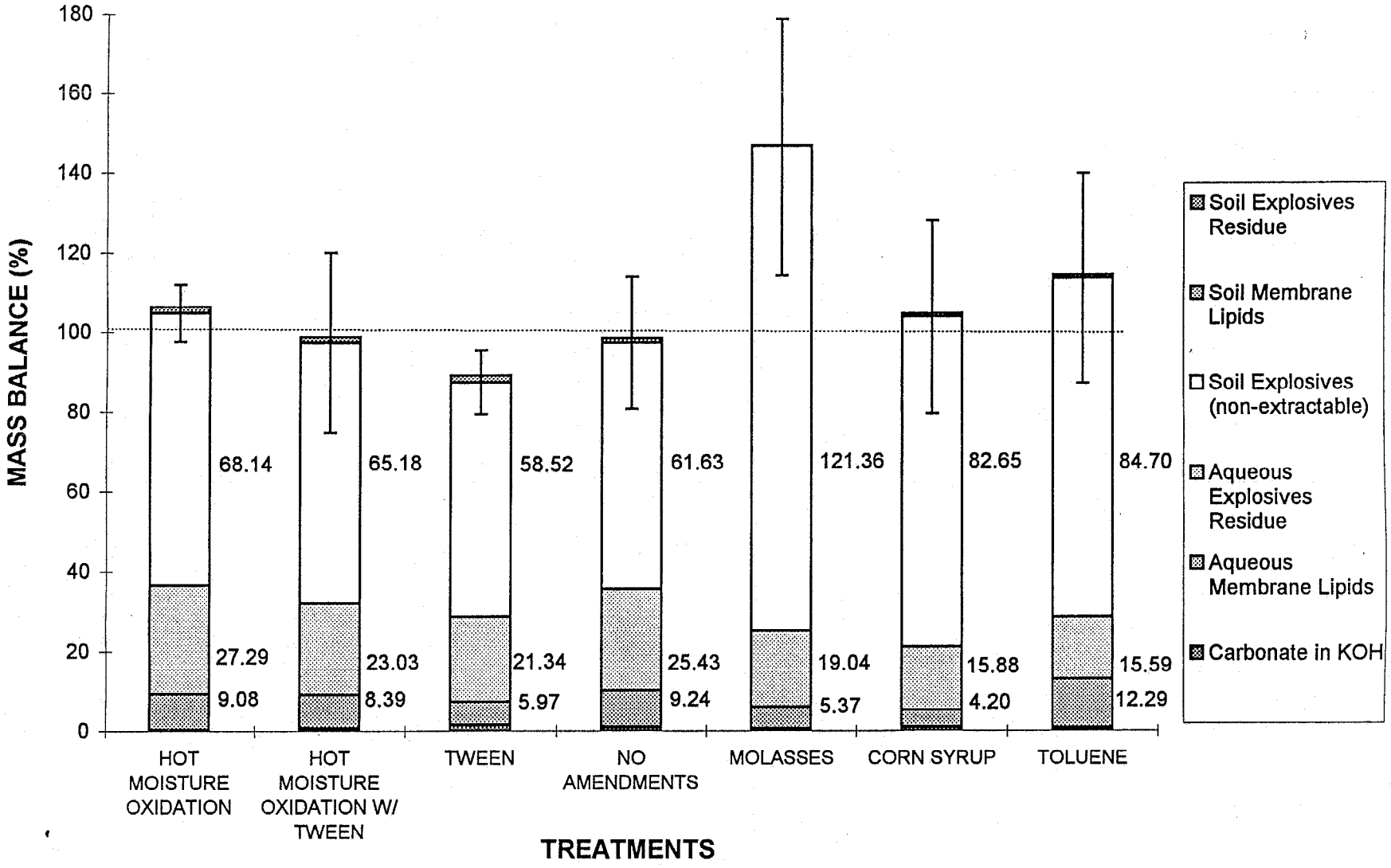


Figure 4. Mass Balance of radiolabeled TNT in microcosms utilizing various treatments simulating aerated bioslurry with native consortia

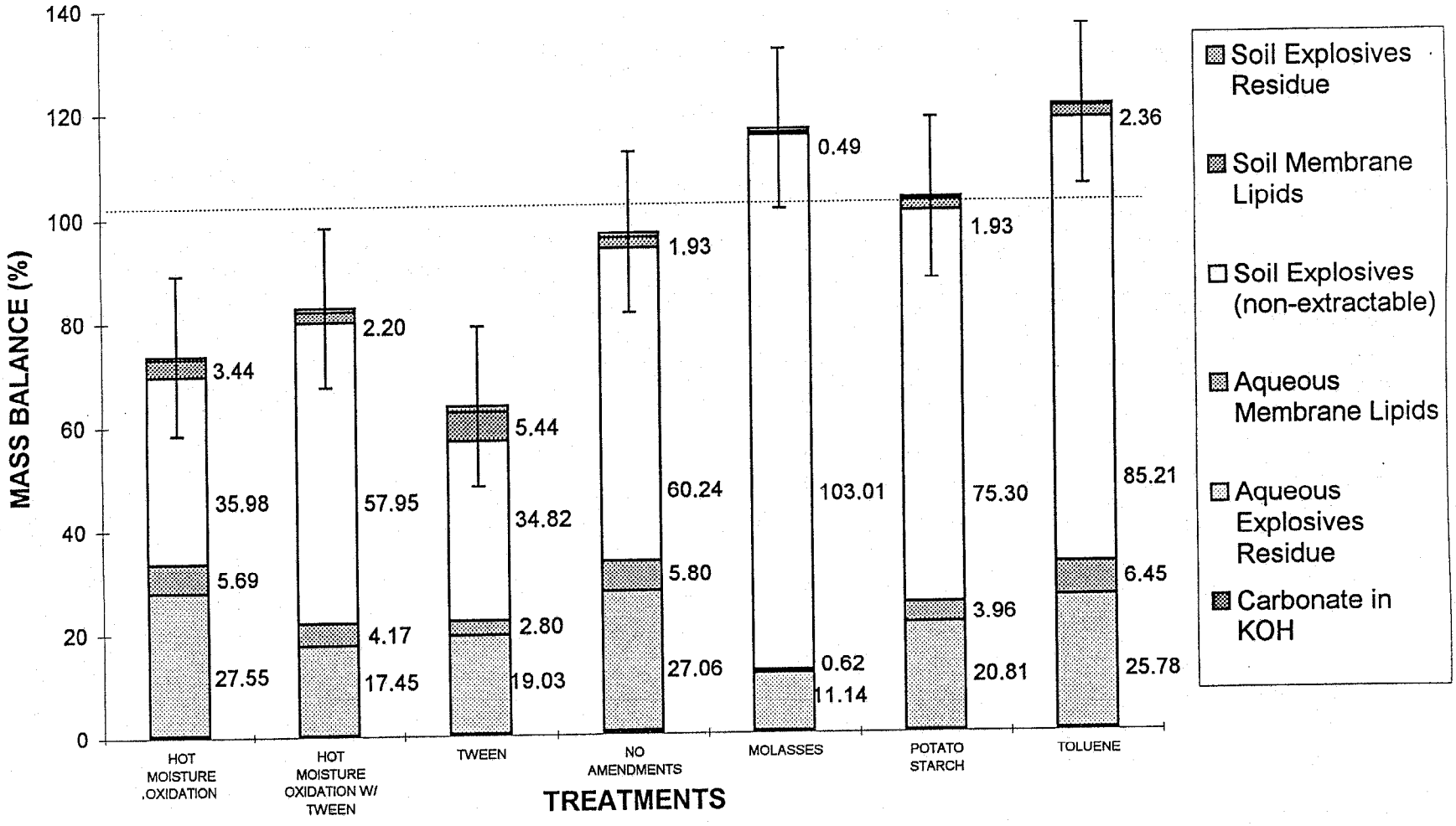


Figure 5. Mass balance of radiolabeled TNT in microcosms utilizing various treatments simulating microaerophilic bioslurry with native consortia

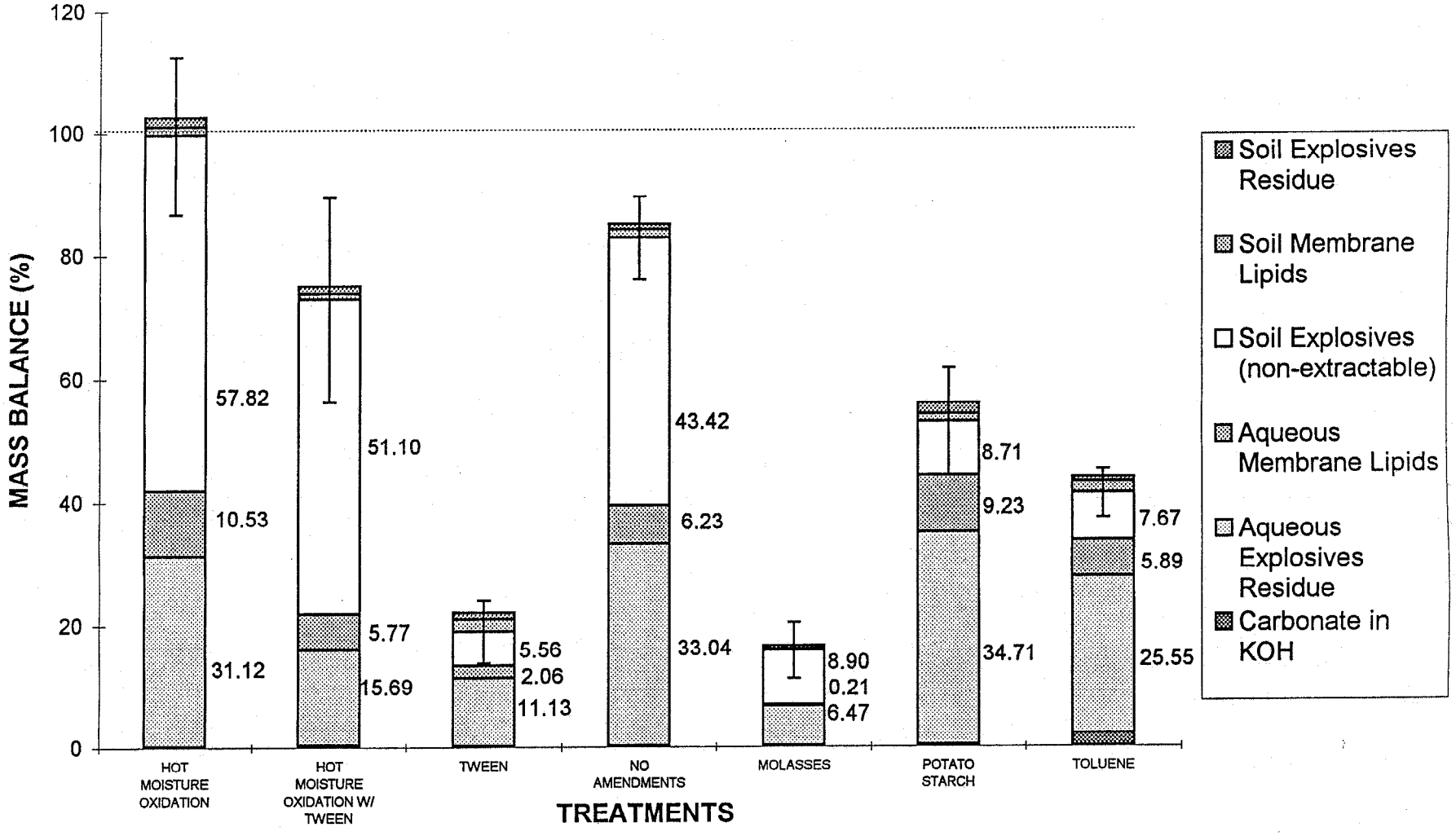


Figure 6. Mass balance of radiolabeled TNT in microcosms utilizing various treatments simulating anaerobic bioslurry with native consortia

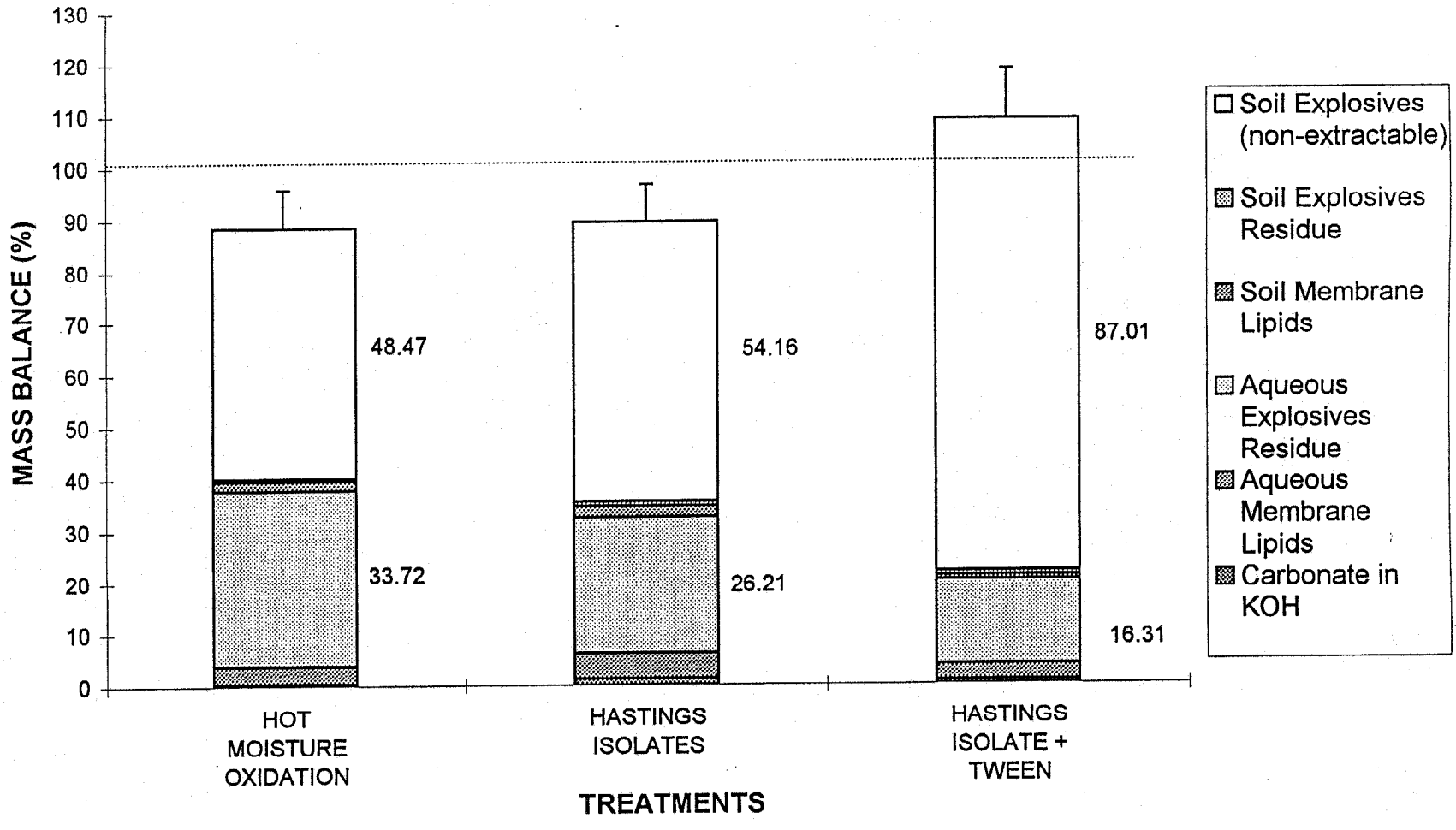


Figure 7. Mass balance of radiolabeled TNT in microcosms utilizing various bioaugmentation treatments simulating aerated bioslurry

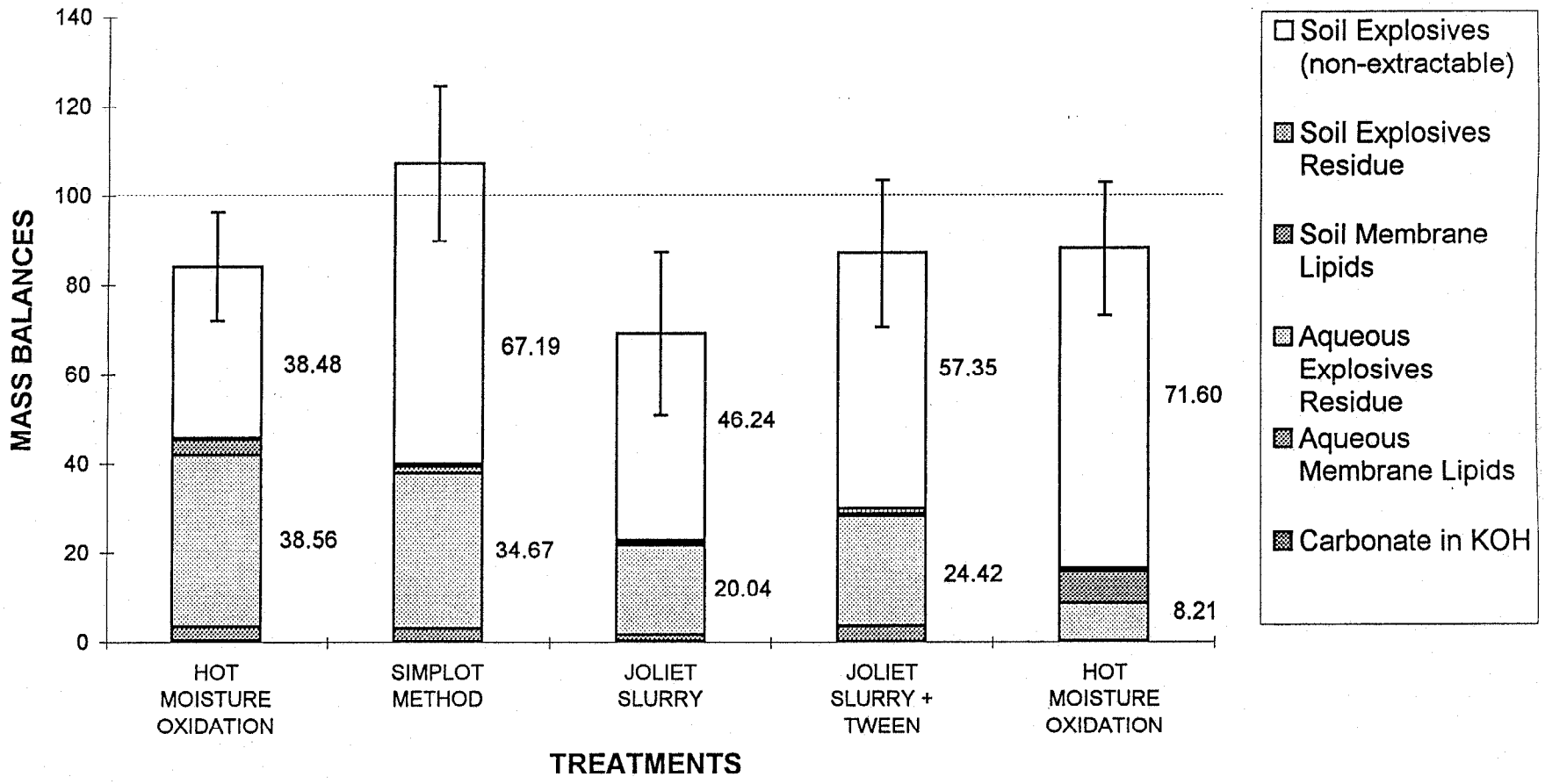


Figure 8. Mass balance of radiolabeled TNT in microcosms utilizing various bioaugmentation treatments simulating anaerobic bioslurry

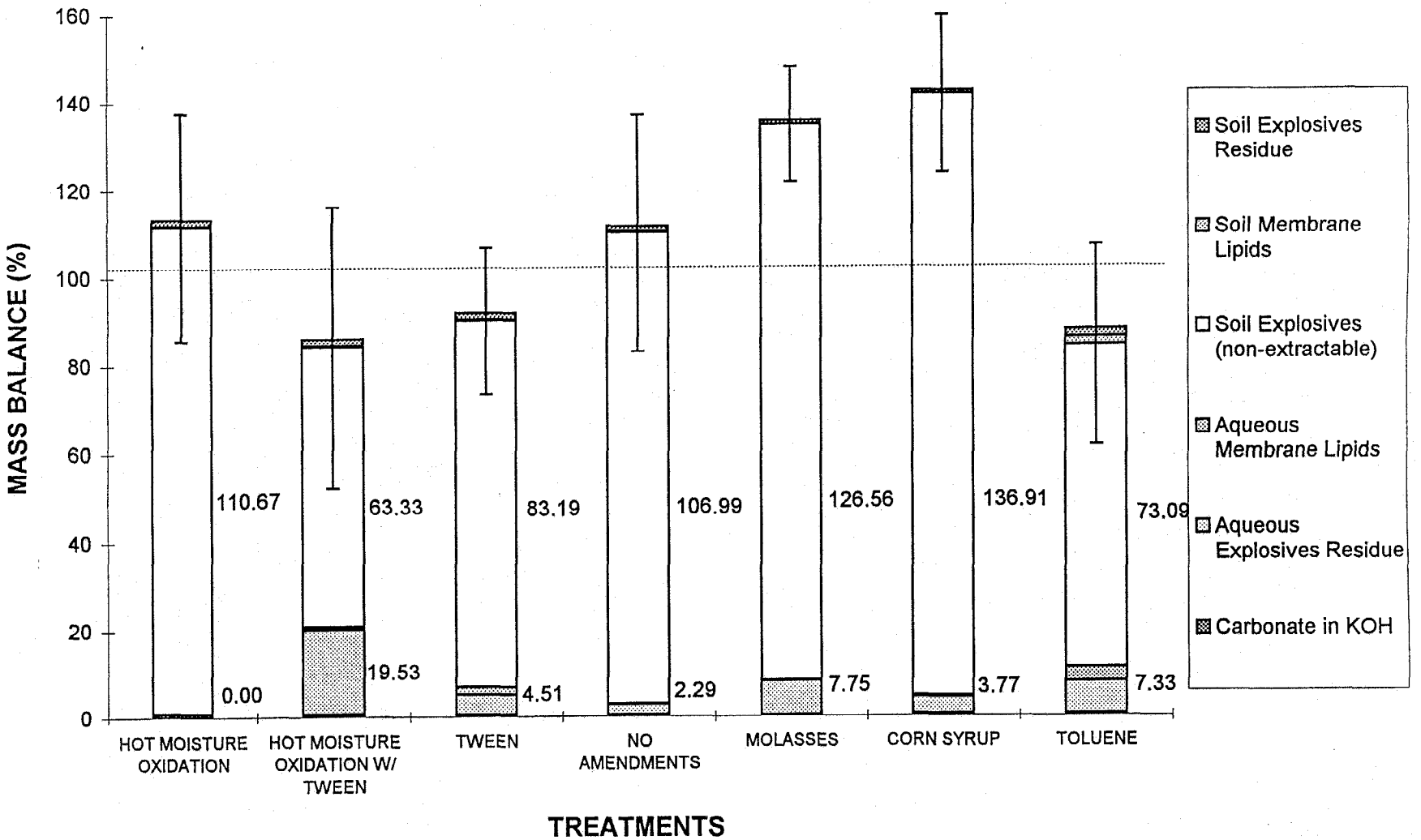


Figure 9. Mass Balance of radiolabeled TNT in microcosms utilizing various treatments simulating aerated biocell with native consortia

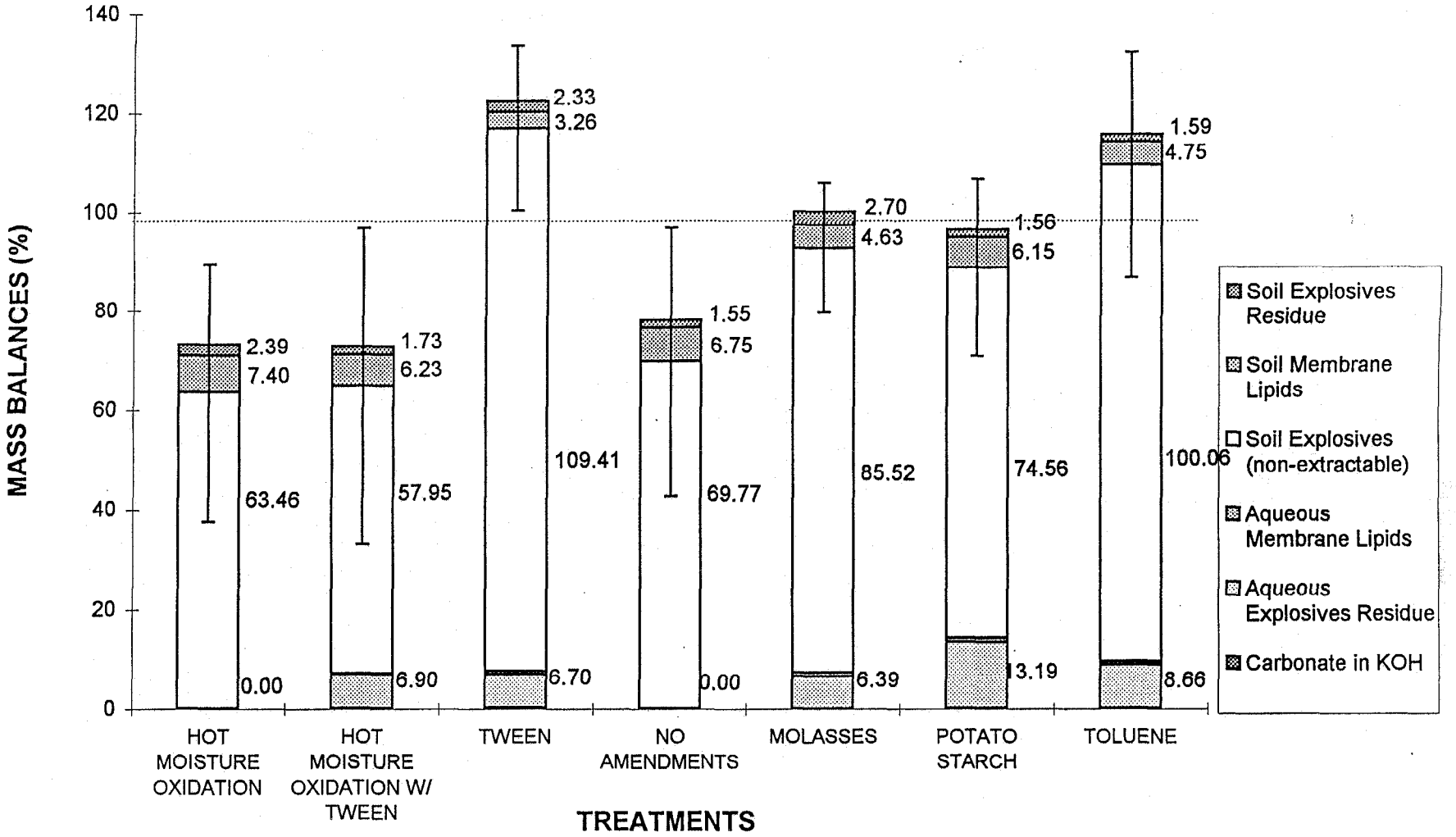


Figure 10. Mass balance of radiolabeled TNT in microcosms utilizing various treatments simulating microaerophilic biocell with native consortia

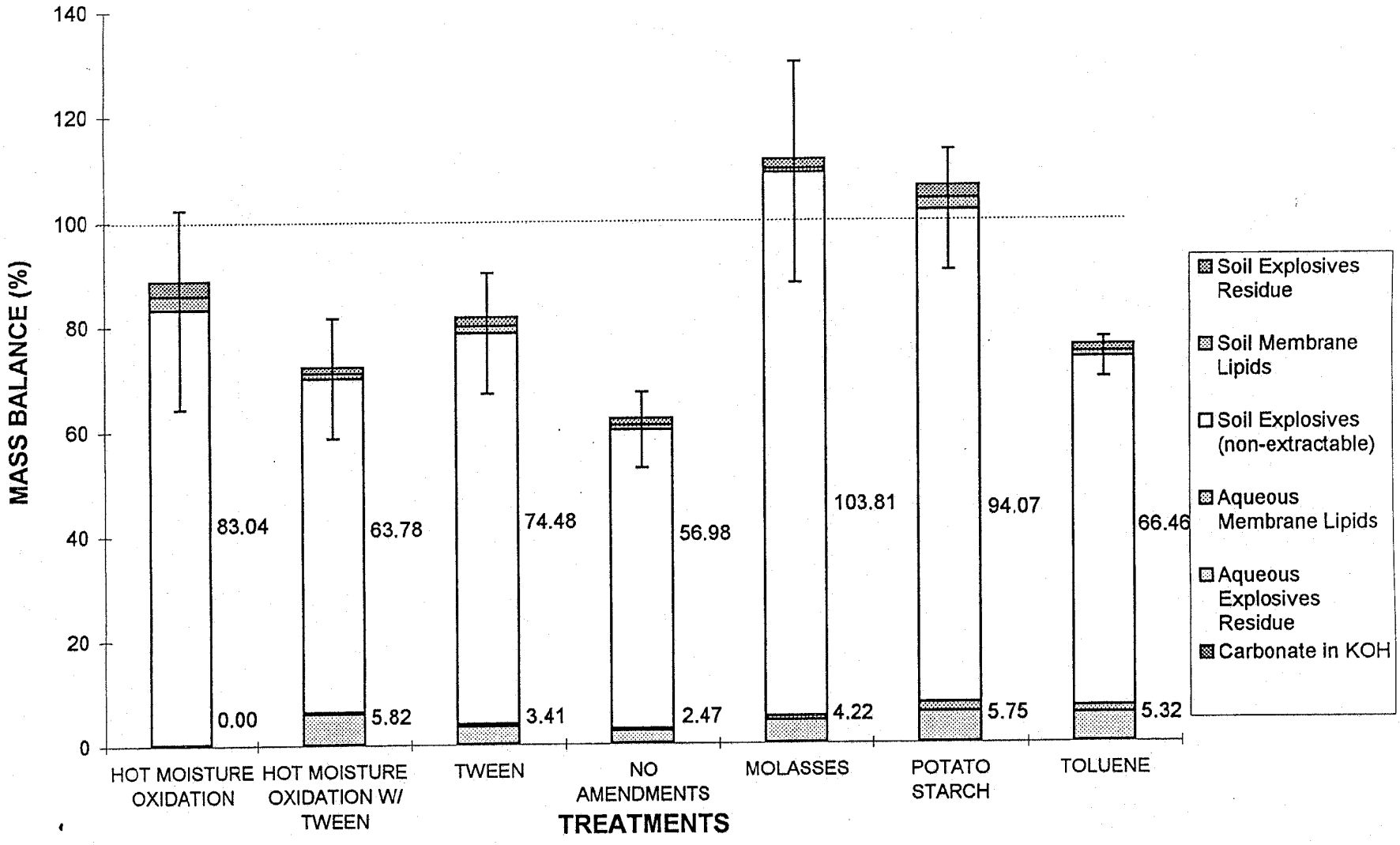


Figure 11. Mass balance of radiolabeled TNT in microcosms utilizing various treatments simulating anaerobic biocell with native consortia

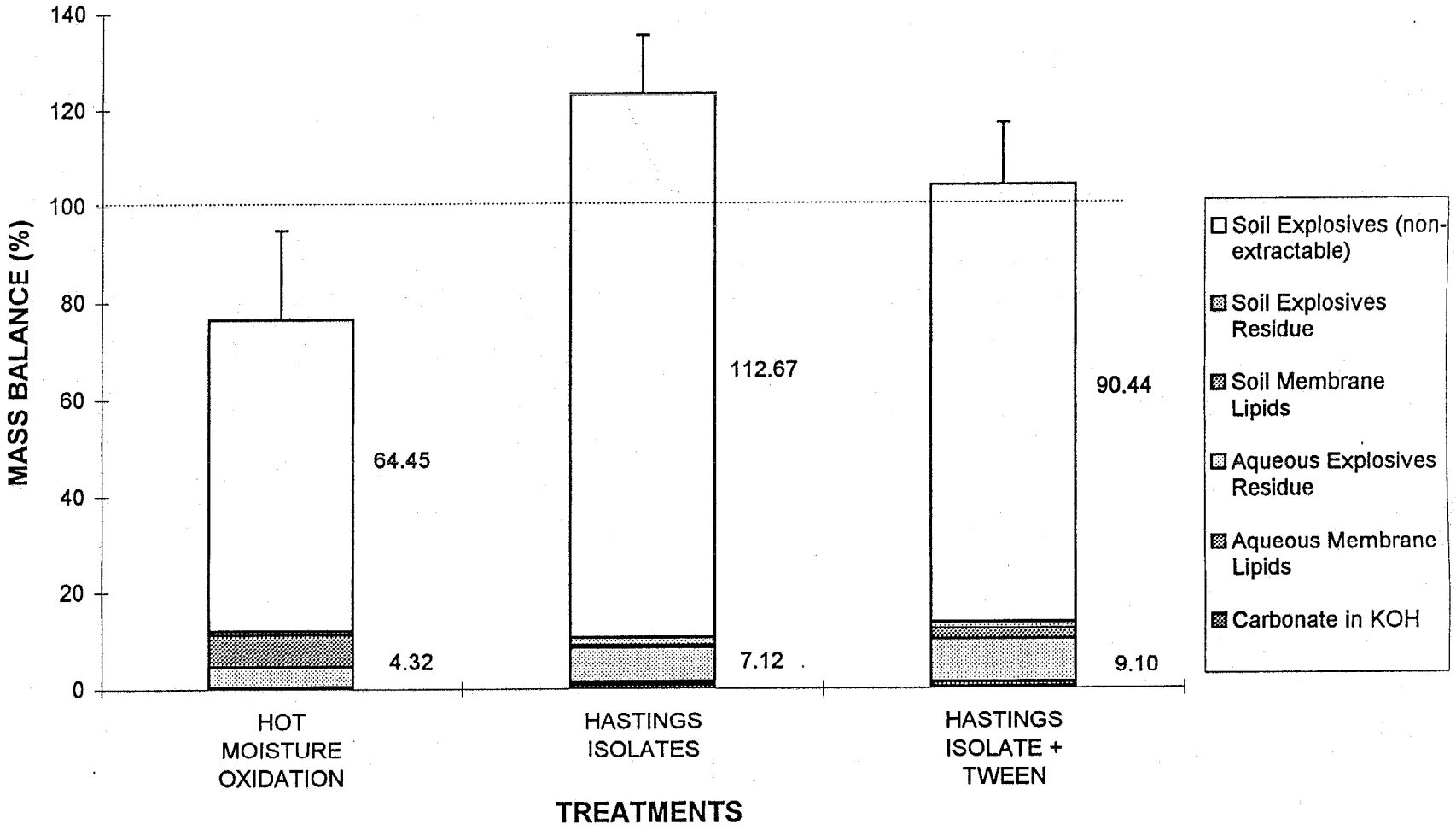


Figure 12. Mass balance of radiolabeled TNT in microcosms utilizing various bioaugmentation treatments simulating aerated biocell

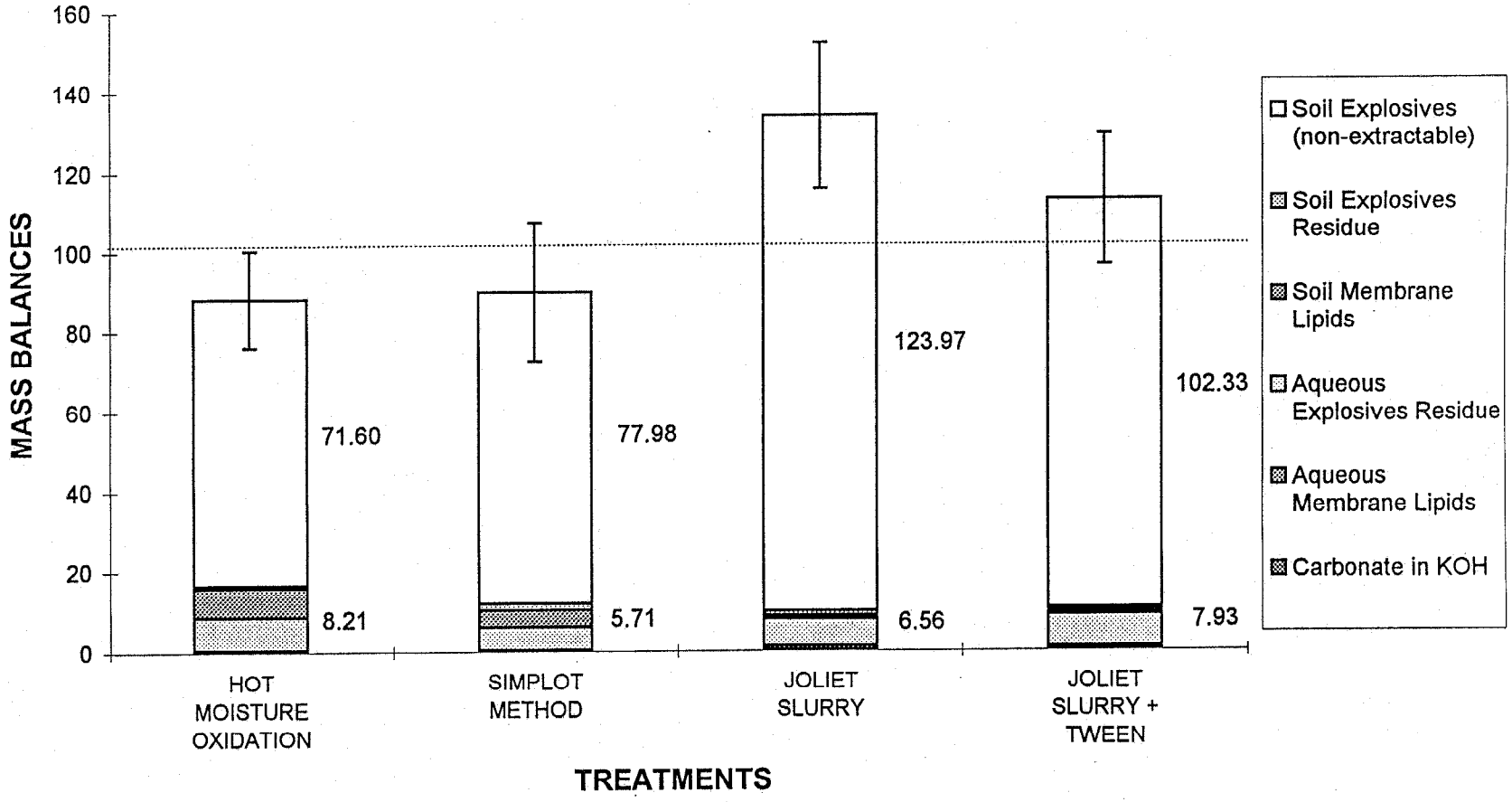


Figure 13. Mass balance of radiolabeled TNT in microcosms utilizing various bioaugmentation treatments simulating anaerobic biocell

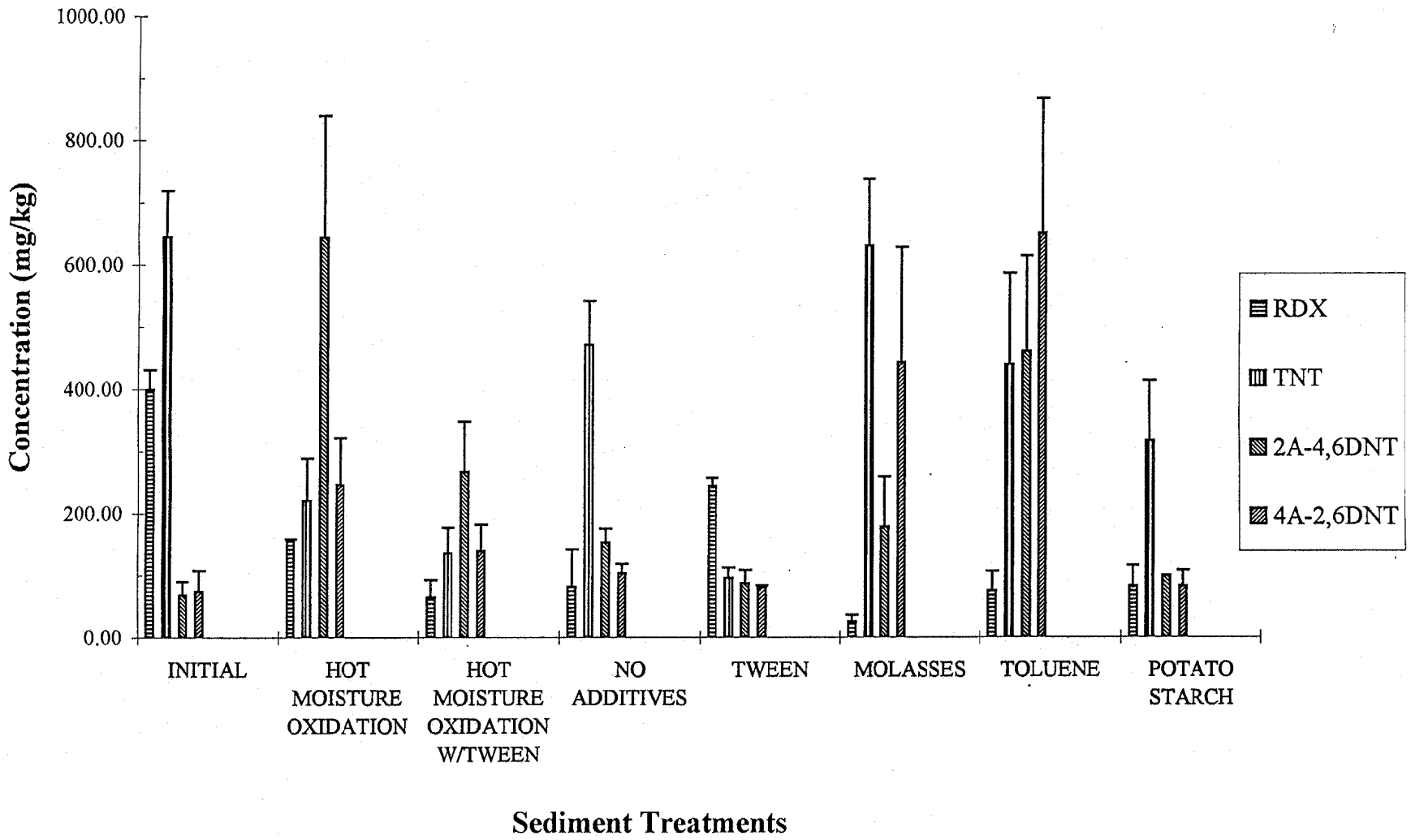


Figure 14. Concentration of non-radiolabeled explosives in soil with native consortia in aerobic 30% bioslurry.

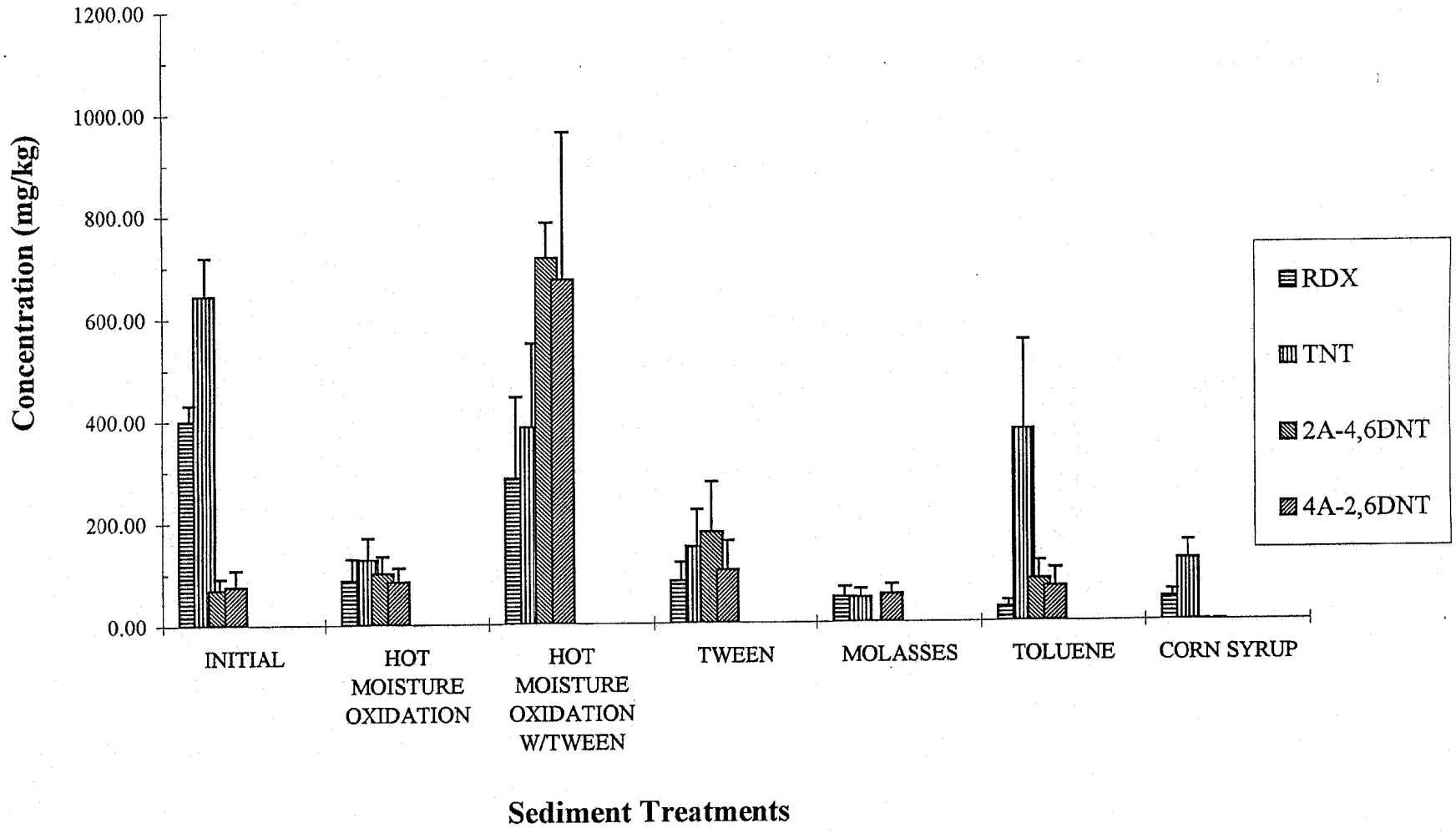


Figure 15. Concentration of non-radiolabeled explosives in soil with native consortia in aerobic biocell

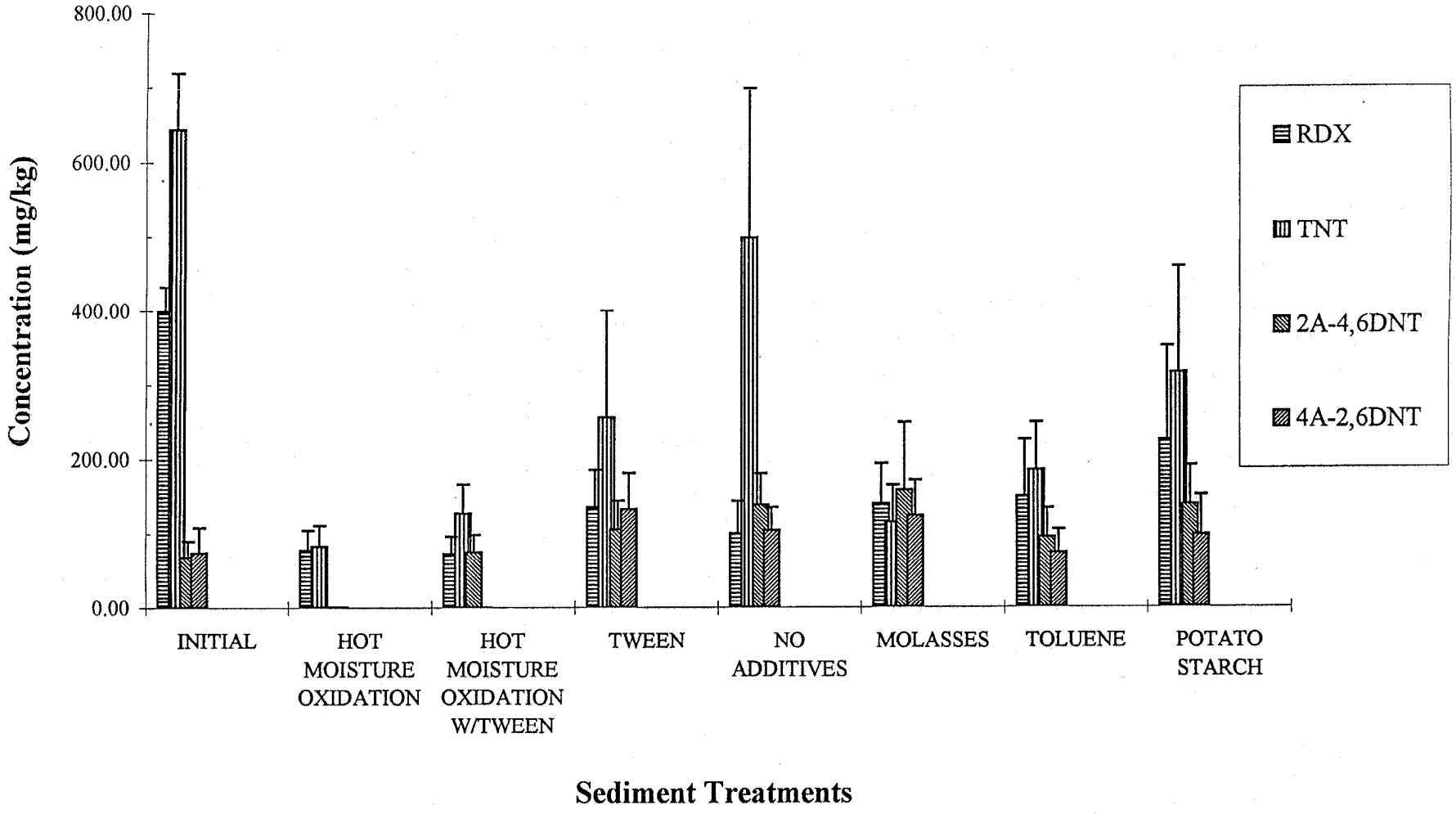


Figure 16. Concentration of non-radiolabeled explosives in soil with native consortia in microaerophilic 30% bioslurry.

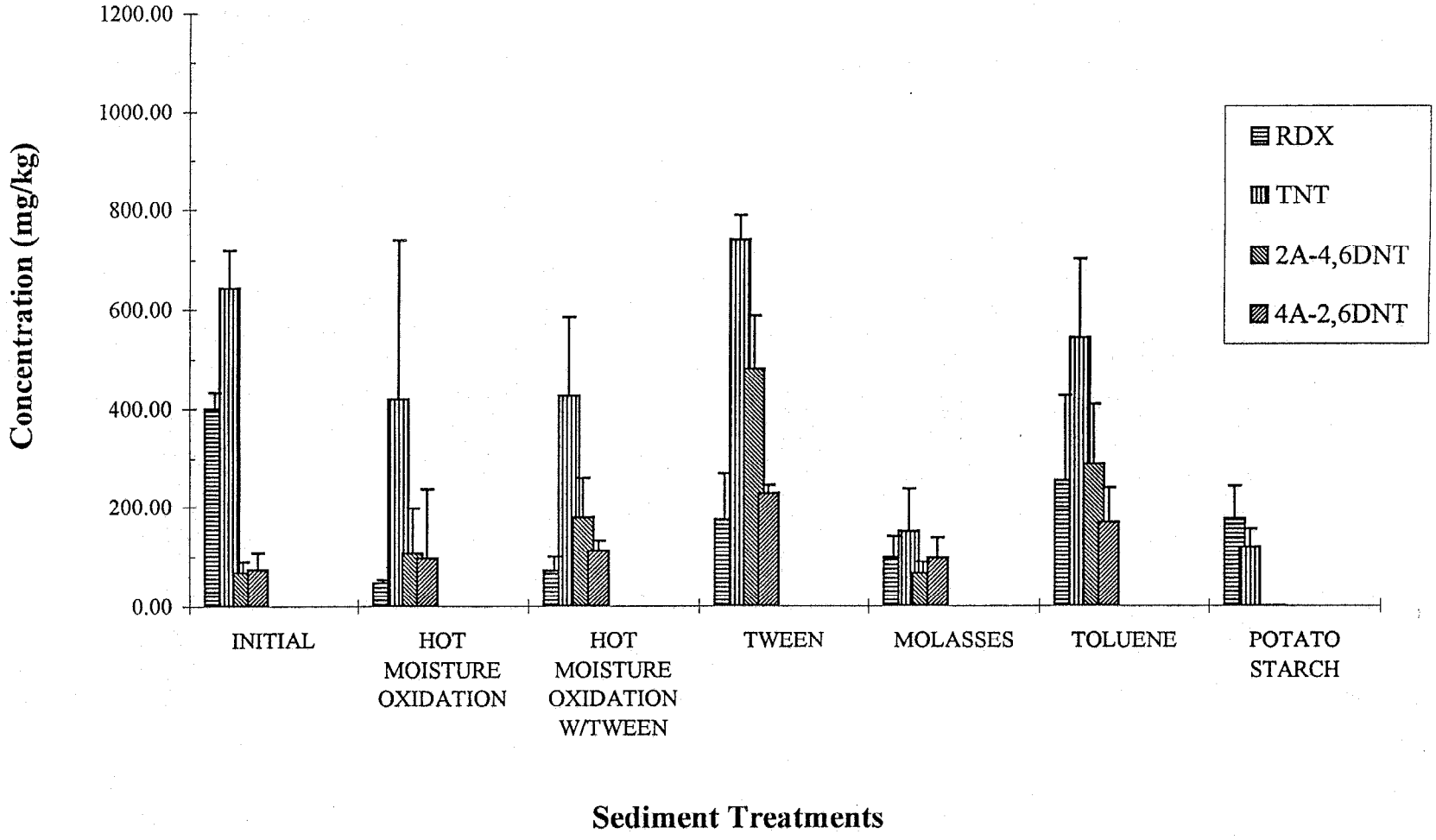


Figure 17. Concentration of non-radiolabeled explosives in soil with native consortia in microaerophilic biocell

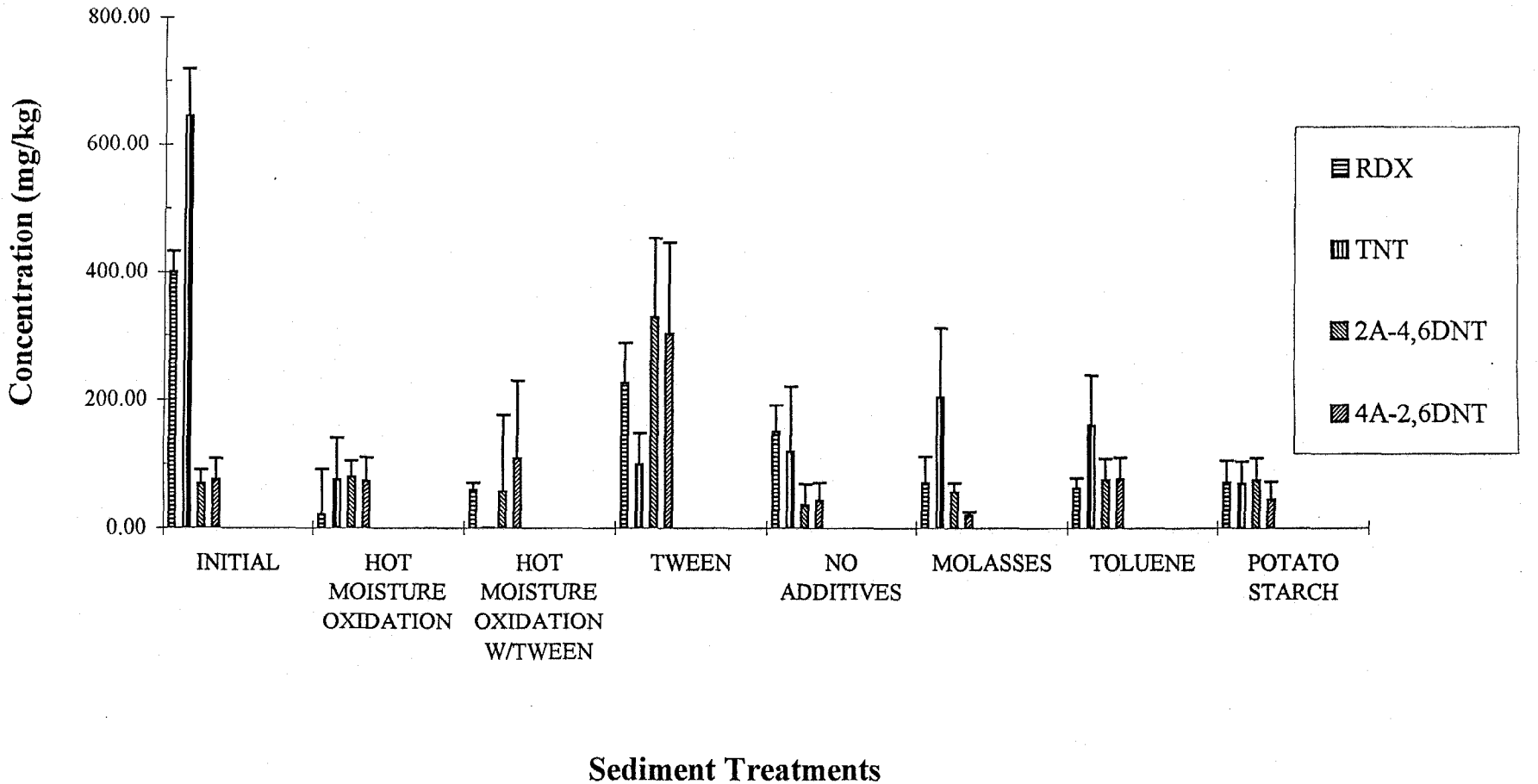


Figure 18. Concentration of non-radiolabeled exposives in soil with native consortia in anaerobic 30% bioslurry

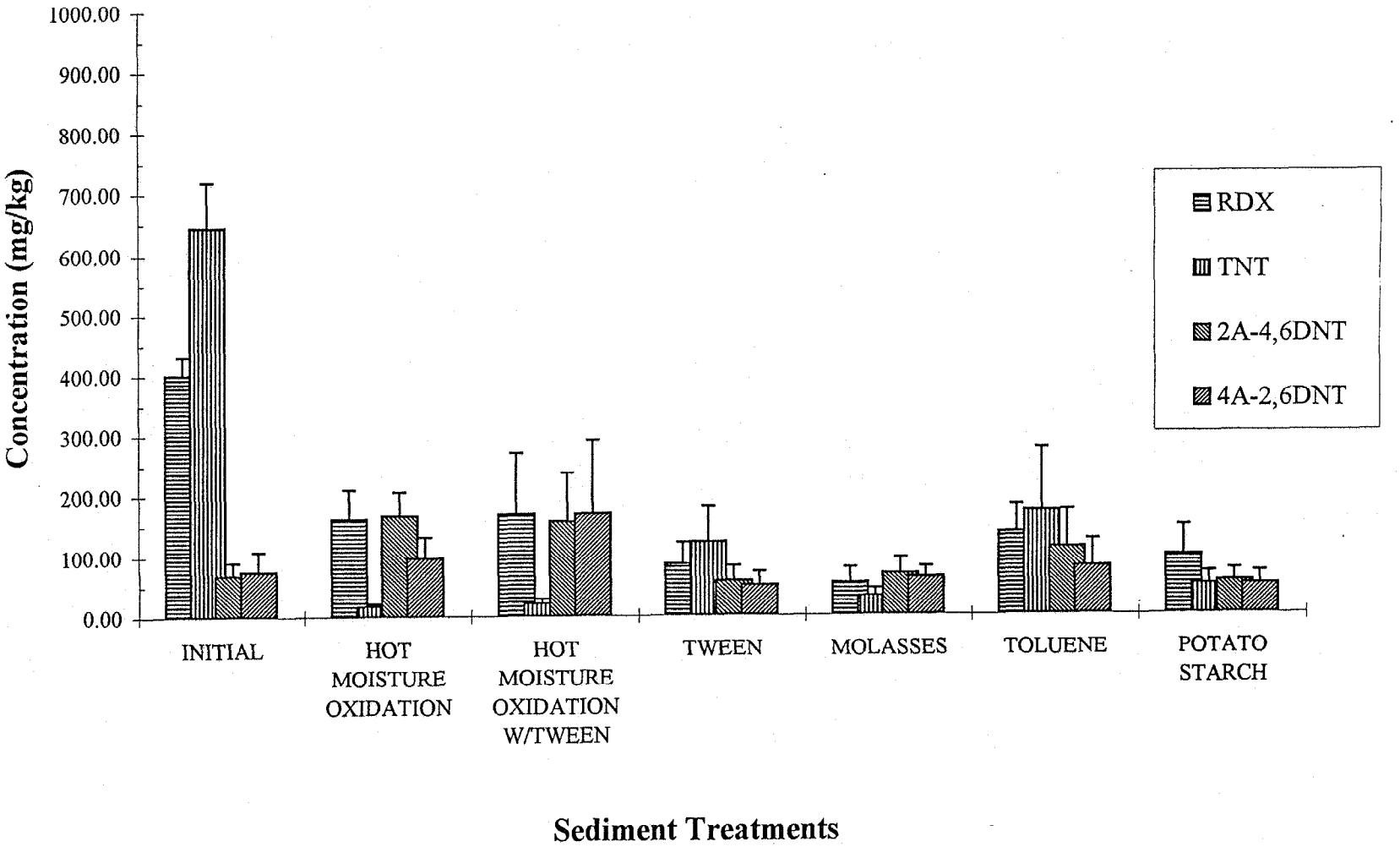


Figure 19. Concentration of non-radiolabeled exposives in soil with native consortia in anaerobic biocell

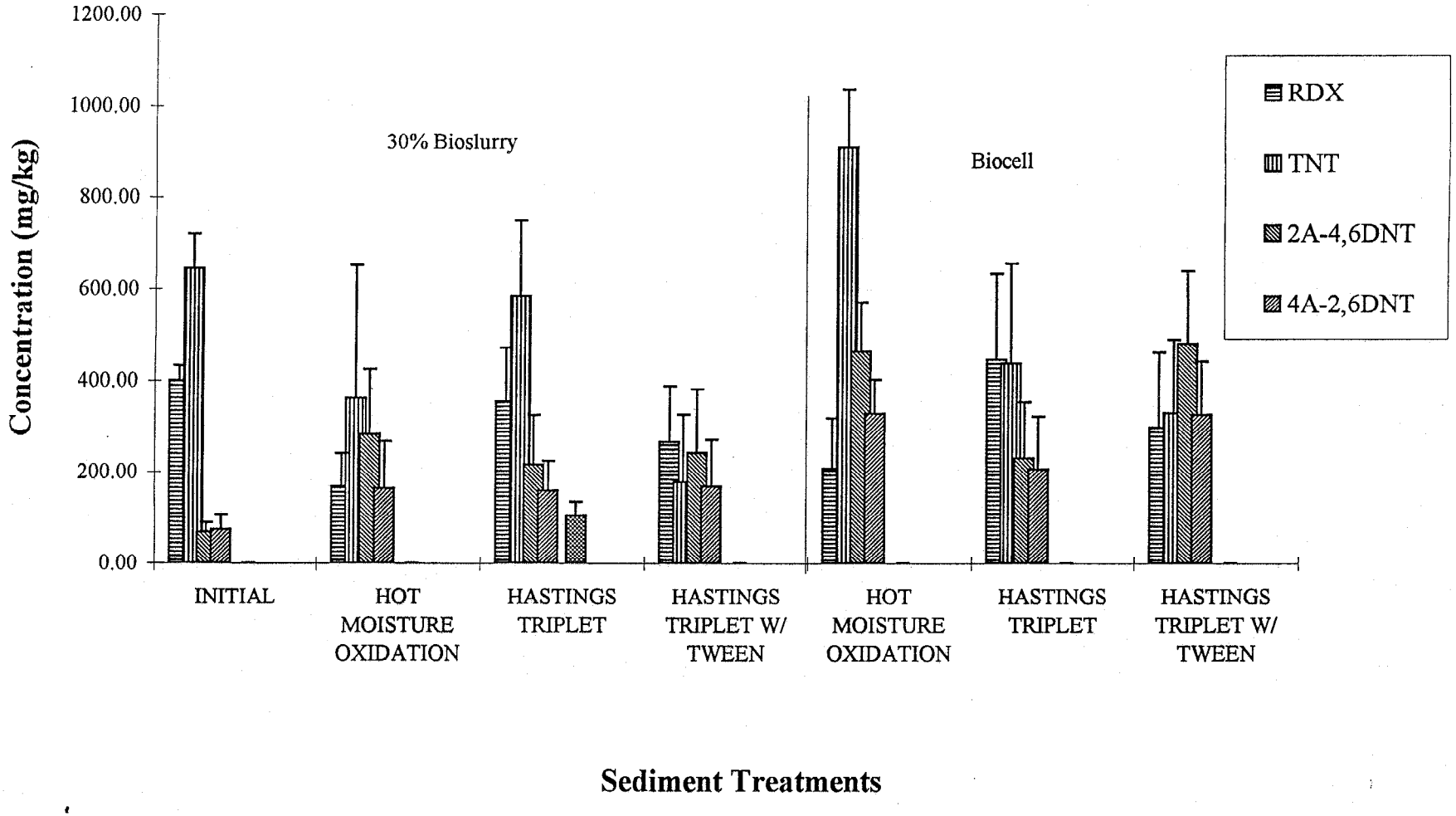


Figure 20. Concentration of non-radiolabeled explosives in a bioaugmented soil in an aerobic 30% bioslurry and biocell

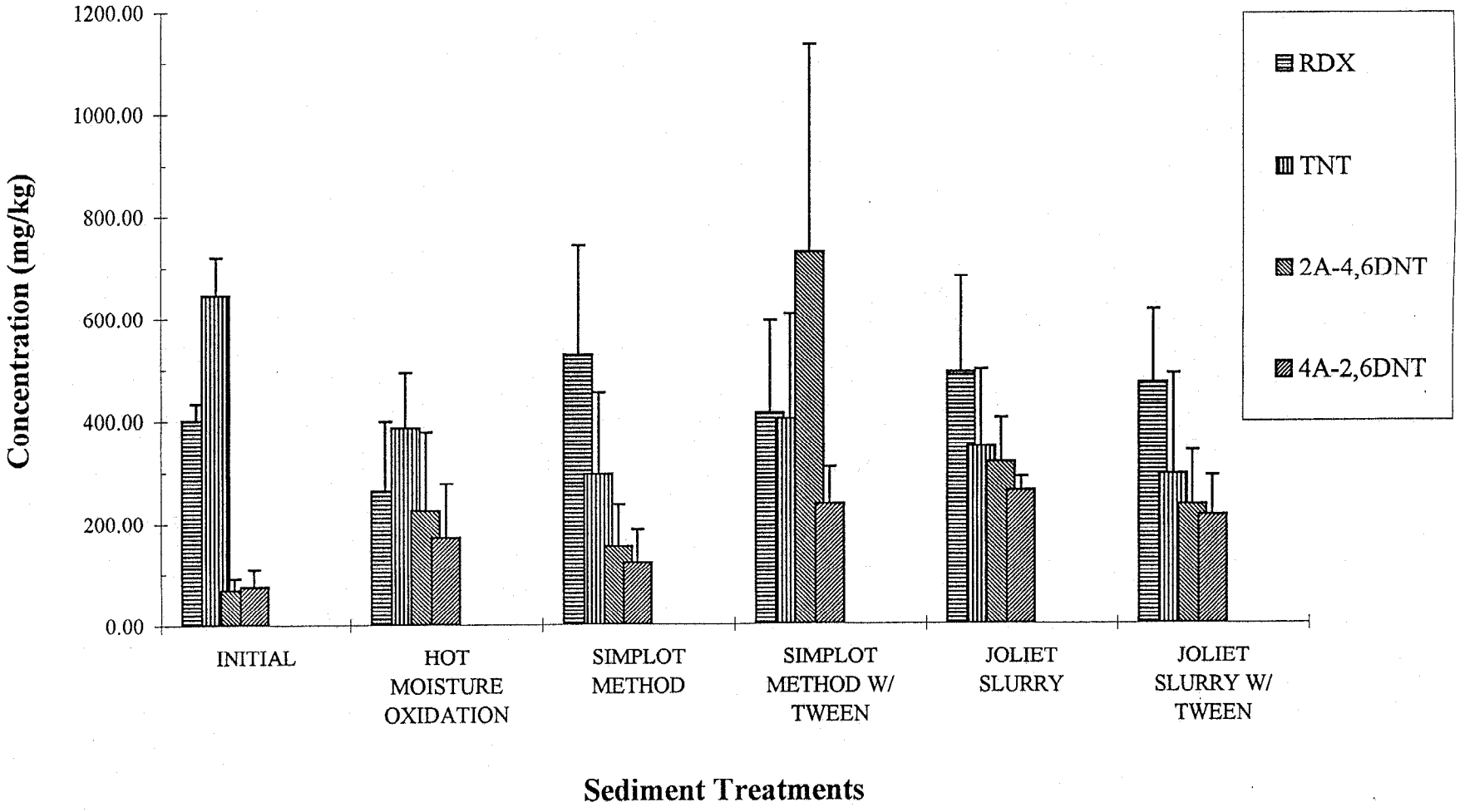


Figure 21. Concentration of non-radiolabeled explosives in a bioaugmented soil in an anaerobic 30% bioslurry

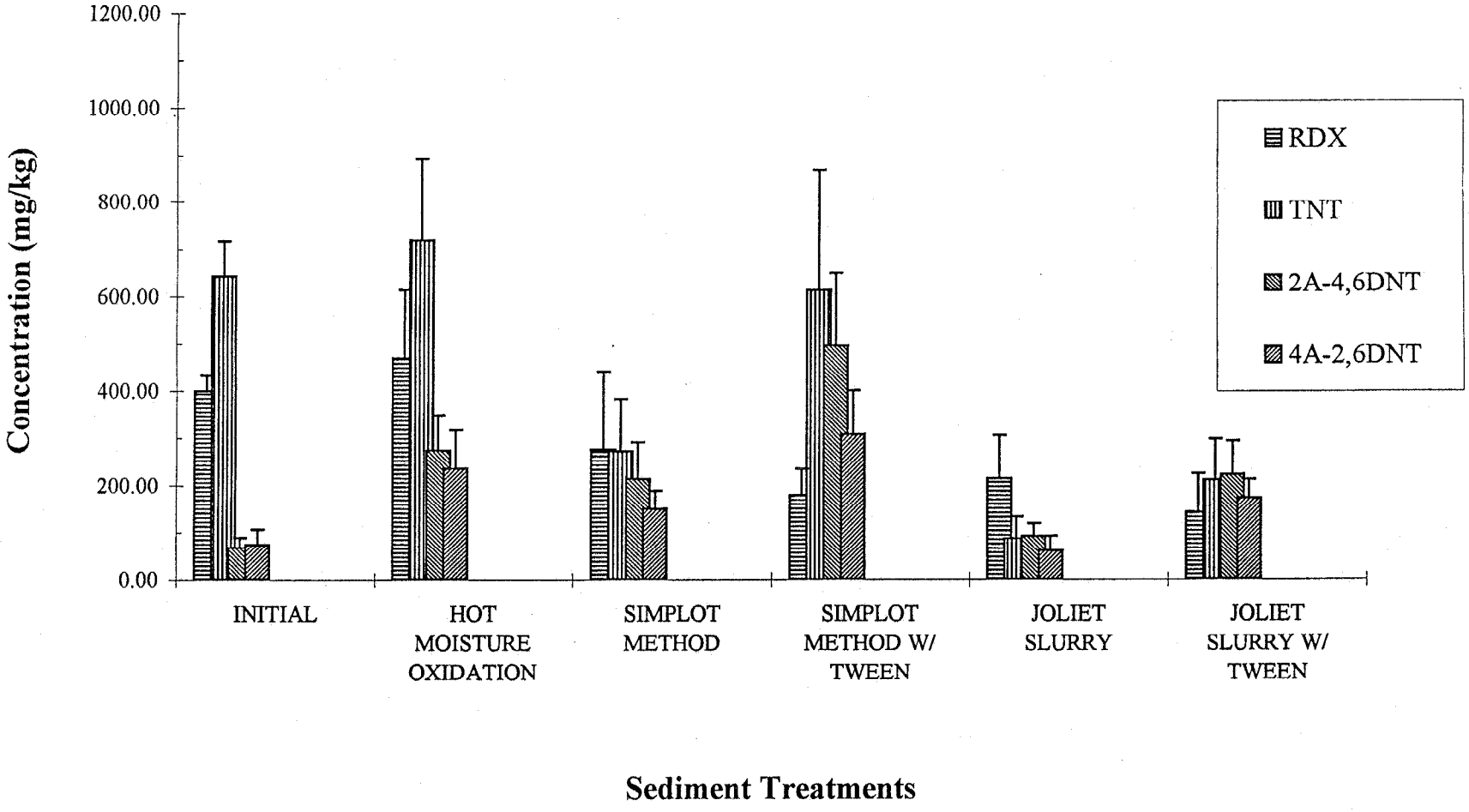


Figure 22. Concentration of non-radiolabeled explosives in a bioaugmented soil in an anaerobic biocell

APPENDIX B
OPTIMIZATION OF SURFACTANT ENHANCED DESORPTION: OPERATIONAL PARAMETERS FOR BIOREMEDIATION OF EXPLOSIVES-CONTAMINATED SOIL (DRAFT)

NOTE: **The attached report is the author's review draft. Per the author's request, the report should not be cited. A final report will be included once available.**

Technical Report
August 1996

US Army Corps
of Engineers
Waterways Experiment
Station

OPTIMIZATION OF SURFACTANT ENHANCED DESORPTION: OPERATIONAL PARAMETERS FOR BIOREMEDIATION OF EXPLOSIVES-CONTAMINATED SOIL

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Author's review draft
Do not cite

Prepared for Headquarters, U.S. Army Corps of Engineers

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Strategic Environmental Research Development Program

Technical Report
July 1996

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Report 2 of a series

Author's review draft
Do not cite

Prepared for U.S. Army Corps of Engineers
Washington DC 20314-1000

Under EIRP Work Unit 31627

Monitored by Environmental Laboratory
U.S. Army Engineer Waterways Experimental Station
3909 Halls Ferry Road, Vicksburg, MS 39180-6199

WES Diagram

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Preface

The work reported herein was conducted by the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, as part of remediation technology assessments by the Naval Facilities Engineering Command (NFEC), Atlantic Division 1824, Norfolk, Virginia. Additionally, this work was also part of the Strategic Environmental Research and Development Program (SERDP), Installation Restoration Research Program (IRRP) and the U.S. Army Environmental Quality Technology Research Program.

Personnel who cooperated in the execution of the study and the preparation of this report include Major Steve Harvey, Principle Investigator; Mr. Todd Richards; Ms. Danea Guimbelot-Polk; and Dr. Mark Zappi, Environmental Restoration Branch (ERB), Environmental Engineering Division(EED).

The report was reviewed by:

The report was prepared under the general supervision of Mr. Norman Francingues, Chief, EED, and Dr. John W. Keeley, Director, EL. At the time of publication of this report, the WES Director was Dr. Robert W. Whalin. and the Commander was COL Bruce K. Howard, EN.

This report should be cited as follows:

> Harvey, S., Richards, T., Guimbellot-Polk, D., and Zappi, M.E. (1996). "Optimization of Surfactant Enhanced Desorption: Operational Parameters For Bioremediation of Explosives-Contaminated Soil", Technical Report, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Introduction

Surfactants are among the most versatile of the products of the chemical industry [Rosen, 1989]. Their uses range from motor oils, pharmaceuticals, detergents, drilling muds for petroleum prospecting, and flotation agents for ore extraction[Rosen, 1989]. Surfactants are also being widely studied for their benefits in the remediation of contaminated soils and waters.

Within biological treatment systems of contaminated soils, there are many complex mechanisms occuring. Generally though, the bioavailability of a contaminant is dependent on its mass transfer and its solubility limit. Surfactants are able to decrease the free energy of a soil slurry system thus reducing resistance to desorption. Additionally, at high concentrations, surfactants self assemble to form micelles. The organic interior of a micelle serves as a hydrophobic area into which contaminants can partition. Thus, surfactants can also increase the aqueous solubility of a contaminant.

In a heterogeneous process such as a soil slurry, the boundary between water and soil is large. This boundary acts to limit the mass transfer rate of contaminant from the soil to aqueous phase. The total amount of contaminant in the aqueous phase will also be limited due by its solubility. A surfactant is a molecule that tends to adsorb on surfaces or interfaces (surfactant is a contraction for surface-active agent) and alters the free energy of surface or interface[Rosen, 1989]. The surfactant contains a hydrophobic group that distorts the structure of water thereby increasing the free energy of the system. Due to this increase in free energy, the surfactant will concentrate at the surface or interface and orient their hydrophobic group to reduce the free energy of the system. The surfactant will reduce the interfacial free energy thus increasing the rate of mass transfer. The surfactant can also reduce the free energy by orienting the hydrophobic groups within as micelles are formed and into which the contaminant will partition. This partitioning can increase the contaminant concentration in the aqueous phase above its solubility limit. Thus, there are two primary mechanisms whereby surfactants reduce the free energy of a system, adsorption at interfaces and micelle formation [Rosen, 1989].

Surfactants have been used to increase the efficiency of pump and treat operations, soil washing and the biological remediation of contaminated soils. Zappi et al. evaluated six nonionic surfactants and acetone for their ability to solubilize TNT from Hastings Park contaminated soil. Zappi et al. found that Tween 80 at 3% (w/w) concentration achieved a concentration of TNT 1.5 times that in water alone. The objectives of this research was to select the most effective nonionic surfactant of three evaluated and its concentration for solubilizing explosives in a bioslurry reactor. The explosives contaminated soil were obtained from the US Navy's Yorktown Naval Weapons Station (WPNSTA).

BACKGROUND

Surfactant Types

Surfactants are amphiphilic compounds which indicates that they are composed of both polar (hydrophilic) and nonpolar (hydrophobic) groups. Surfactants are generally classified according to the structure of the hydrophilic portion of the molecule. A negatively charged head group is an anionic surfactant, a positive charged head group is a cationic surfactant, both positive and negatively charged head group is amphoteric, and a head group with no charge is a nonionic surfactant. In general, the order

of solubilizing power for hydrocarbons and polar compounds appears to be as follows: nonionics > cationics >anionics for surfactants with the same hydrophobic chain length [Rosen, 1989].

Anionic Surfactants

Anionic surfactants are manufactured and used in greater volume than all other type of surfactants [Porter,1994]. The negatively charged hydrophilic portion of an anionic surfactant is usually associated with a cation such as sodium. In an aqeous solution, the molecule ionizes to yield the sodium cation and the anionic surfactant. As such, the anionic surfactants are sensitive to electrolyte concentration which may lead to surfactant precipitation. The precipitation of anionic surfactants by electrolytes limits their usefulness in the remediation of some aqueous systems. Anionic surfactants are widely used as detergents and are the surfactant of choice in enhanced oil recovery research [Pennell, 1996].

Cationic Surfactants

Cationic surfactants posses a postive charge and as such are strongly adsorbed to soil minerals which are generally negatively charged. Since the cationic surfactant is positively charged it is not practicle for use in the remediation of contaminated soils as they are generally negatively charged. Additionally, many cationic surfactants are toxic to bacteria and fungi which could result in the elimination of native consortial in soil [Pennell, 1996].

Nonionic Surfactants

Nonionic surfactants are widely used in food products, pharmaceuticals, and detergents. The hydrophilic group of a nonionic surfactant consists of either hydroxyl groups or an ethylene oxide (EO) chain. By varying the number of EO groups during manufacture, the hydrophobicity of the surfactant can be manipulated. The solubility of EO groups is due to the hydrogen bond between water and the EO group [Porter,1994]. As the number of Amphoteric EO groups increase, the solubility of the surfactant increases. Nonionic surfactants are not susceptible to the same electrolyte and pH limitations as the anionic and cationic surfactants. However, nonionic surfactants are susceptible to temperature changes. As the temperature increases, the surface tension will increase for a given surfactant concentration.

Amphoteric Surfactants

Amphoteric is derived from the Greek amphi meaning both and used to describe surfactants which have both positive (cationic) and a negative (anionic) group. In acidic solutions they form cations, in alkaline solutions they form anions, and in a middle pH range the molecule has two ionic groups of opposite charge (zwitterionic) [Porter, 1994]. In general, amphoteric surfactants have not been used in remediation applications as they are expensive, tend to adsorb strongly, and are produced in small quantities[Pennell, 1996].

Micelle Formation

At low concentrations, surfactant molecules exist as monomers and adsorb onto surfaces or at interfaces. As the surfactant concentration increases, the monomers eventually provide a monolayer coverage of the surface. The adsorption of monmers leads to physical changes in water, the most distinct being the decrease in surface tension. Surface tension will decrease with increasing surfactant concentration until a minimum is reached. At this minimum, the surface will have a monolayer coverage and the surfactant concentration is known as the critical micelle concentration. At concentrations above the

CMC, there are no longer any sites available for adsorption. At this point the surfactant molecules will orient their hydrophobic and hydrophilic groups so that like groups are together and combine to form micelles. At the CMC, surfactants will form clusters, with the hydrophobic tails oriented within and the hydrophilic portion oriented outward. Since the CMC of a surfactant indicates complete monolayer adsorption the it represents the lowest concentration to achieve the maximum benefit [Porter, .1994].

The adsorption of the surfactant at an interface can be described by the Gibbs adsorption equation:

$$\Gamma_i = -\frac{d\gamma}{du_i} \qquad (1)$$

where Γ = surface excess concentration of component *i*

$d\gamma$ = change in surface tension

du_i = change in chemical potential of any component *i*

At equilibrium,

$$du = RTd\ln ai \qquad (2)$$

where R is the gas constant, T is the absolute temperature (Kelvin), and a_i is activity of component in the bulk phase. Thus,

$$d\gamma = -\Gamma RTd\ln Ci \qquad (3)$$

a plot of surface tension versus the log plot of surfactant concentration will show a rapid decrease in the surface tension of water as surfactant concentration approaches the CMC. At the CMC, the surface tension will no longer decrease as monolayer coverage has been achieved. The CMC is reflected on the plot by an inflection point.

Micelle Structure

The number of surfactant molecules in a micelle is known as the aggregation number[4]. The aggregation number of nonionic micelles varies between 40 and 400 (at room temperature)[Datyner, 1983]. The major types of micelles are[Rosen, 1989]:

- small, spherical structures (<100 aggregation number)
- elongated cylindrical, rodlike micelles with hemispherical ends
- large, flat lamellar micelles
- vesicles - spherical structures consisting of lamellar micelles arranged in concentric spheres.

Locus of Solubilization

In general, the locus of solubilization varies with the material and its interaction with the surfactant. Solubilization is postulated to occur at the following sites in the micelle[Rosen, 1989]:

- at the micelle-solvent interface (1)
- between the hydrophilic head groups (2)
- in the palisade layer (between the hydrophilic group and the first few carbon atoms of the hydrophobic group) (3)
- further in the palisade layer (4)
- the inner core of the micelle (5)

Solubilization in nonionic surfactants is postulated to occur between the hydrophilic head groups (polyoxyethylene groups)[Rosen, 1989].

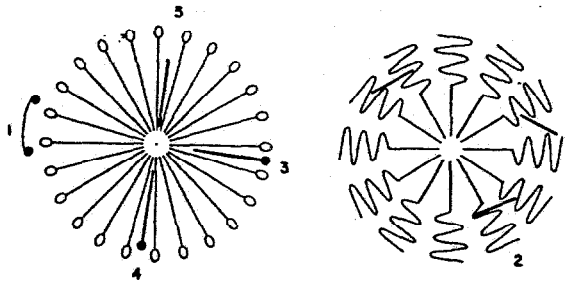

Figure 1-1. Locus of solubilization of material in a surfactant micelle. Numbers in figure correspond with location description above [Rosen, 1989].

Equilibrium Models

The sorption of contaminants onto solid surfaces are typically modeled using isotherms. The more commonly utilized forms are the linear, Langmuir, and Freundlich. When the isotherm is nonlinear, a sorption mechanism other than hydrophobic partitioning is operative and the two most common models are the Freundlich and Langmuir. In this work, we are concerned with the desorption of explosives from contaminated soils. Hence, we will attempt to model explosive desorption with these isotherms.

Linear

The linear isotherm is the most commonly used model in part due to its simplicity. The sorbate-sorbent interaction is a linear relation described as follows:

$$S_i = K_{d(i)} C_i \qquad (4)$$

where S_i represents the adsorbed concentration, $K_{d(i)}$ is the distribution coefficient of the contaminant, and C_i is the aqueous phase concentration. Based on earlier work, explosives have been postulated to be best modeled by near-linear isotherms [McGrath,1995].

Freundlich

The Freundlich model describes the nonlinear sorption relation as an exponential function of solute concentration as follows:

$$S_i = K_F C^b_F \qquad (5)$$

Typically, data is fitted to the linearized form of equation 5:

$$\ln S = \ln K_F + b_F \ln C \qquad (6)$$

K_f may be obtained from a log-log graph by raising the intercept to the power of ten and b may be obtained by the slope of the line from a log-log plot.

Langmuir

The Langmuir isotherm describes the adsorption process as the reversible formation of a monolayer. Once the monolayer sites are filled, sorption would be weak and the adsorbed concentration represents a maximum, adsorbate concentration [McGrath]. The Langmuir model is:

$$S = \frac{K_L S_{max} C}{1 + K_L C} \tag{7}$$

Langmuir isotherm parameters are typically obtained by fitting adsorption data to a linearized form of equation 7:

$$\frac{1}{S} = \frac{1}{S_{max}} + \frac{1}{K_L S_{max}}\left(\frac{1}{C}\right) \tag{8}$$

for which a plot of 1/S versus 1/C has an intercept of $1/S_{max}$ and slope of $1/(K_L S_{max})$.

Effect of Surfactant on Bioavailability

A conceptual model has been proposed to incorporate the possible mechanisms of biodegradation and solubilization of contaminants [Pennell, 1996]. Figure 1-2 shows the distribution of the hazardous organic contaminant (HOC) and surfactant between solid, liquid, and micellar phases is shown in figure 1-2 (steps 1-4). Biotransformation of the HOC and surfactant are shown in steps 5 and 6. The partitioning of the HOC (step 4) will be influenced by the surfactant concentration. Below the CMC, the adsorption of surfactant onto the solid phase may also increase the concentration of the HOC (step 1). Above the CMC, the HOC will partition into the micelle (step 3) and result in an increase in HOC solubility. At sub-CMC concentrations, surfactant monomers can alter the cell membrane and enhance biotransformation of the HOC (steps 5 & 6) [Pennell, 1996].

Effects of various parameters on nonionic surfactants

pH

As expected, pH variation affects cationic and anionic surfactants the most as solid surfaces will become more positively charged as the pH decreases and negatively charged as the pH increases. For nonionic surfactants, the ether linkages in the polyoxyethylene chains can become protonated at low pH, yielding a positively charged head group.

Temperature

As the temperature increases, the polyoxyethylene head group dehydrates which decreases its solubility[Rosen, 1989]. At high enough temperature, the surfactant molecule can precipitate out of solution causing a milky appearance. This is known as the cloud point, which is in the range of 50 C.

Ionic Strength

The presence of electrolytes in solutions causes the CMC to change with the effect being most pronounced for cationic and anionic surfactants, followed by amphoteric and nonionic [Rosen, 1989]. The added electrolyte can reduce the electrostatic repulsion between hydrophilic groups of ionic surfactants, thus lowering the CMC [Porter, 1994]. Electrolyte effects on nonionic surfactants are considerably less as the hydrophilic head is not charged.

2 Materials and Methods

Soil Preparation Methods

The soil used for all studies was a composite sample from three different sites at the Naval Weapons Station at Yorktown, Virginia. The soil was homogenized and mixed prior to shipment to the Waterways Experiment Station. The 55-gallon drums were stored in the Hazardous waste research center (HWRC) cooler at 4 degrees Celsius (°C). Soil was further homogenized by mixing and sieving. Soil was sieved using a U.S. Standard #4 sieve (4.76 mm opening). The sieve removed gravel, rocks, twigs, and other debris. The homogenized soil was subsequently analyzed for explosive compounds such as HMX, RDX, TNB, DNB, TNT, 4A-DNT, 2A-DNT, 2,6-DNT, and 2,4-DNT using EPA method 8330.

Surfactants Examined

The surfactants used in this study were all nonionic and included the following: Tween 80, Simple Green®, and Witconol SN 120. The Witconol SN 120 was a gift from Witco Corporation, Houston, Texas. Tween 80 and Simple Green® were purchased from respectively: Sigma Chemical Corportation, and Sunshine Makers, Inc., Huntington Harbour, California. Characteristics of the surfactants are listed below:

Table 2.1 Surfactant Characteristics

Surfactant	CMC mg/l	Formula weight	Specific Gravity	biodeg-radeable	HLB	Cloud point	Aggregation number (N)	Molecular formula
Tween 80	15	1309.6	1.08	y	15	unk	110	$C_{64}H_{124}O_{26}$
Witconol SN 120	120	570	1.03	y	14.3	185-197F	105	unk
Simple Green®	43	unk	1.02	y	unk	unk	unk	unk

Methods for Determination of Critical Micelle Concentration (CMC)

Solution Preparation

Stock solutions of 20% surfactant by weight were prepared for Tween® 80. Serial dilutions were performed to prepare each standard to be measured. Prior to use, all glassware were cleaned with chromic acid followed by a triple rinse of distilled, deionized (DDI) water and allowed toair dry. Standards were stored in 120 milliliter amber sample jars. Sample jars were Quality Control (QC) grade purchased from Environmental Sampling Supply (ESS). Stock solutions and standards were prepared immediately prior to each experiment to assure surfactant solution quality.

Methods for CMC determination in DDI water

Surface tension was measured with a Fisher Surface Tensiomat Model 21. The Standard Test Method for Surface and Interfacial Tension of Solutions of Surface-Active Agents, ASTM D1331-89, was the protocal used for this study. The Fisher Surface Tensiomat employs a du Nouy type platinum-iridium ring

for surface tension measurements. Measurements were conducted in an environmentally controlled laboratory with the temperature maintained at 22 °C. Air movement and vibration in the laboratory were eliminated. Time sensitivity for surface tension was tested and an equilibrium time selected. Jars containing the samples were shaken to ensure consistent initial conditions. After shaking, the sample was placed into a petri dish and allowed to equilibrate for a period of 20 minutes prior to measurement. Between samples, the ring was rinsed in acetone and then placed into a flame until red-hot to remove any residual surfactant on the ring. Three experiments, each with fresh surfactant solution were performed to confirm the CMC in DDI water.

Methods for CMC determination in a soil slurry

The homogenized soil contaminated with explosives from the Yorktown site were utilized for this experiment. A representative sample was removed and its moisture content determined by constant weight within a change of 0.05 percent per minute using a Denver Instruments moisture analyzer. A slurry was formed in 250 milliliter polypropylene centrifuge bottles using surfactant solution and soil to achieve a 30% (weight soil/weight water) solids content. A soil slurry without surfactant was prepared as the blank.

The soil/surfactant slurries were then placed on a reciprocating box shaker at 180 excursions per minute for 24 hours. After shaking, the bottles were removed and centrifuged for 20 minutes at 5,000 revolutions per minute (rpm). The supernatant from the centrifuged samples were then decanted and filtered through a 0.45 micron, type A/E, Gelman filter followed by a 0.22 micron, type Acetate Plus, MSI filter. Surface tension of the filtered samples was measured as outlined above.

Methods to Examine the Desorption of Explosives from Contaminated Soil

Methods for Batch Desorption Experiments

Batch desorption experiments were conducted to determine the optimum surfactant and its concentration in desorbing explosives from soils. Surfactants examined in the batch desorption experiments included Simple Green®, Tween® 80, and Witconol® SN 120. Homogenized soil was measured for moisture content by constant weight within a change of 0.05 percent per minute using a Denver Instruments moisture analyzer. All glassware was cleaned with chromic acid followed by a triple rinse with DDI water. Sterility was assured through the use of an autoclaved with soil and associated glassware. The autoclave operated at a temperature of 225 °F and a pressure of 15 psig for a 20 minute actual autoclave cycle time. Surfactant solutions were filter sterilized using 0.22 micron MSI type Acetate Plus filters. The soil was mixed with prepared surfactant solutions at a 30 percent ratio of soil to water (w/w) in 250 milliliter polypropylene centrifuge bottles. Three separate centrifuge bottles were prepared for each concentration of surfactant standard. DDI water was used as the blank for this experiment.

Soil samples were equilibrated on a reciprocating box shaker at 180 excursions per minute for 24 hours. The soil slurries were centrifuged at 5000 revolutions per minute for 20 minutes. After centrifuging, the decanted liquid was filtered through 0.45 and 0.22 micron filters. Liquid samples were placed in 40 milliliter (Quality Control grade) amber sample vials. The soil phase was removed from each centrifuge bottle and stored in 40 milliliter amber sample containers. Liquid and soil samples were immediately sent to the Environmental Chemistry Branch (ECB) for analysis. The samples were analyzed using the USEPA method 8330 for measurement of nitroaromatics and nitramines by High Performance Liquid Chromatography (HPLC).

Methods for Sequential Batch Desorption Experiments

Only Witconol® SN 120 and Tween® 80 were studied in the sequential batch desorption experiment. Surfactant solutions were filter sterilized as above. Glassware used in the experiment was cleaned with chromic acid and then triple rinsed with DDI water. Glassware and soil was autoclaved as above. Thirty percent soil slurry solutions (w/w) were prepared in 250 milliliter polypropylene centrifuge bottles by combining soil with the surfactant standard solutions. Triplicate centrifuge bottles for each surfactant concentration were prepared.

Soil slurries were allowed to equilibrate for 24 hours on the reciprocal shaker. Samples were centrifuged and the supernatants were decanted, filtered, and stored in amber vials at 4 °C. Fresh surfactant solution was then added to each centrifuge container and each sample equilibrated on the reciprocating box shaker for 24 hours. This process was repeated a total of four times. At the end of the fourth day liquid and soil samples were collected. All of the soil and aqueous phase samples were analyzed for explosives using USEPA method 8330.

Methods to Examine Desorption Kinetics

The objective of this experiment was to measure the rate of explosives desorption. One system consisted of soil and DDI water and a second system consisted of soil and 3 percent Tween® 80. Homogenized soil was analyzed for moisture content and three samples collected for explosives analysis. Soil collected for explosives analysis was stored in amber sample containers at 4 °C. Thirty percent soil slurries (w/w) were prepared in 3.785 liter (1gallon) jars.

The jars were sealed and placed in a mechanical tumbler. Liquid samples were collected via disposable syringes at the following elapsed times: 0.5, 1, 2, 6, 12, 24, 48, and 96 hours. Samples were placed in 40 milliliter polypropylene centrifuge tubes and centrifuged at 12,000 rpm for 15 minutes. The supernatant was decanted and filtered through 0.45 and 0.22 micron filters. Filtered samples were placed in amber sample containers and stored at 4 °C. The remaining soil phase was homogenized and triplicate soil samples were collected at 96 hours. All collected liquid and soil samples were analyzed by the USEPA method 8330 for explosives concentrations.

3 Results and Discussion

Determination of Critical Micelle Concentration

Surface tension equilibrium

The first objective was to determine equilibrium of the surfactant solution for measurement of surface tension. Different concentrations of surfactant solutions were analyzed at various time intervals to determine equilibrium. The time span denotes how long the Denouy ring was in solution prior to surface tension measurement. As expected the age of the surface in the sample has a great effect on the measured value of surface tension. Differences between values measured at ages of 2 minutes and those measured at 15 to 40 minutes were 15 dyne per centimeter lower. For example, at a concentration of 10 milligrams per liter Tween 80, the surface tension was 62 dyne per centimeter at two minutes versus 50.4 dyne per centimeter at twenty minutes. Greater discrepancies were noted at lower concentrations of the surfactant.

Figure 3.1 is a plot of surface tension versus surfactant concentrations for various time intervals of measurement. The time interval represents the amount of time that was allowed for the sample to come to equilibrium prior to reading the surface tension. From the plot it is evident that surface tension values are approximately the same after a equilibration time of 15 minutes. In order to be conservative a value of 20 minutes was chosen as the time to reach equilibrium in all subsequent surface tension measurements. Thus, in all experiments involving the measurement of surface tension, the sample was allowed to equilibrate for 20 minutes prior to the reading.

Determination of Critical Micelle Concentration (CMC) in Distilled Deionized Water

In order to verify the accuracy of the tensiomat and procedure it was necessary to compare experimental surface tension values for DDI water and acetone to those published in literature. The reported value for the surface tension of pure water at a temperature of 22 °C is 71.1 dyne per centimeter. The average value obtained was 71.3 dynes per centimeter.The published value for acetone is 24 dynes per centimeter and 23.9 dyne per centimeter was obtained. The experimental values we obtained compare quite favorably with those found in the literature.

Once the time to reach equilibrium had been established it was now possible to determine the critical micelle concentration of Tween 80 in DDI water. As previously discussed, a plot of surface tension versus the logarithm of surfactant concentration will show an inflection point at the CMC. This plot is shown in figure 3.2, and two distinct linear regions can be observed; the sub-CMC region and the supra-CMC region. The sub-CMC region (sloping line) is indicative of surfactant monmers in solution and the supra-CMC (line that is linear) is indicative of micelles in solution. The relation of the CMC on the graph to the change in the slope of the curve can be seen quite clearly in figure 3.2. In other words, the CMC is that concentration at which the inflection point occurs.

In order to calculate the CMC, the plot of surface tension versus concentration was iteratively regressed using different data sets for each linearization until the best fit was found for each region.The equation for each line and their respective residual values are shown in table 3.1 (concentration, C, is mg/L). Both equations were then set equal to each other and the critical micelle concentration determined as the unknown value. Based on this approach, a value of 47.2 milligrams per liter for the CMC was

calculated in this manner at a surface tension of 40.6 dyne per centimeter. A value of 15 milligrams per liter for the CMC was reported by the manufacturer.

Table 3.1. Linear equations for sub-CMC and surfactant supra-CMC regions and their r^2 values

Region	Equation of the line for surface tension (dynes/cm)[1]	r^2
Sub-CMC	$\gamma = -6.3838\ln(C) + 65.25$	0.977
Supra-CMC	$\gamma = -0.4475\ln(C) + 42.37$	0.837

[1]Note C is in mg/l

The difference in the published CMC value for Tween 80 and that obtained by use of the tensiomat is probably due to two primary factors. The first is surfactant sorption onto glassware. Due to its vary nature, the surfactant will accumulate at interfaces and orient its hydrophobic portion away from water. Thus, surfactant will be lost due to sorption. In conducting the CMC experiments, consistent surface tension values were obtained at each surfactant concentration measured. The surface tension values were within the one percent variation mandated by the standard method. However, variations greater than one percent were noted when comparing values obtained from different replications of the experiment. This error is probably due to surfactant sorption onto glassware during solution preparation which would result in less surfactant in solution and a higher surface tension reading. This increase in surface tension due to surfactant sorption can be readily demonstrated by the plot provided in figure 3.3. This is a plot of surface tension versus surfactant concentration with a line that has been forced to pass through a surface tension of DDI water only (72.1 dyne/cm) from the inflection point. A CMC of near 15 mg/l is obtained and the increase in surface tension due to surfactant sorpion can be readily viewed.

The Effect of Filtration on Surface Tension in Surfactant Solutions

Since one of the objectives of this work was to determine the CMC in a soil slurry, it was necessary to determine the effect on surface tension due to filtering a soil slurry. Samples would be filtered prior to surface tension measurements to remove any colloidal matter that may cause error. The concern was that the filter would serve as an interface that surfactant could partition to as the sample was filtered. Surfactant losses would then introduce error into soil slurry surface tension measurements. Table 3.2 shows the difference is surface tension values for samples before and after filtration with a 0.45 um filter. For surfactant concentrations between 5 to 50 mg/l (which is the CMC), filtration does cause a significant difference in the measured surface tension value (alpha = 0.1). Between this range of surfactant concentration, it appears that surfactant sorption onto filter paper results in surface tension measurements that are higher than actuality. At concentrations greater than the CMC (50 mg/l), surface tension is the same after filtration. This is due to the fact that at concentrations greater than the CMC, any losses due to sorption are not significant. The surfactant concentration is in excess and have oriented themselves into micelles. Due to the fact that surfactant concentration in soil slurries will approach the percent range , it appears that filtration will not introduce error in surface tension measurements.

Table 3.2. Effect of Filtering Sample on Surface Tension: Loss of Surfactant

Concentration (mg/l)	Surface Tension	
	Unfiltered (dyn/cm)	Filtered (dyn/cm)
0.10	72.0	72.1
0.50	71.5	71.8
1.00	63.6	63.7
5.00	53.8	59.3
10.00	51.1	56.1
15.00	49.3	54.1
20.00	45.7	49.8
25.00	44.5	47.1
50.00	42.1	44.7
100.00	40.4	41.9
1000.00	38.7	389
10000.00	37.3	37.3
100000.00	35.7	35.9

Determination of CMC in Soil Slurry with Tween 80

Three separate batch desorption studies of soil and various Tween 80 surfactant doses were performed to determine the CMC. New stock and standard solutions of the surfactant were prepared and combined with the soil into the 30 percent soil slurry for each experiment. After shaking, centrifuging, and filtering, the aqueous phase of the slurry was measured for surface tension.

The control consisted of DDI water and soil to account for the effect of soil on surface tension. A significant reduction in surface tension was noted in the soil slurry control as compared to the surface tension of pure water. The surface tension of pure water was 71.3 dyne per centimeter and that of the soil slurry control (no surfactant) was 59.3 dyne per centimeter.

Results of the experiment along with the linear regression output have been plotted as shown in figure 3.4. A significant reduction in surface tension did not occur until the surfactant concentration increased to 1000 milligrams per liter. Since reduction of surface tension is due to surfactant in monomeric form orienting at interfaces, it appears that at concentrations less than 1000 mg/l, the surfactant was sorbing onto the soil. The surface tension significantly decreased between surfactant concentrations of 1 and 3%. Beyond 3 percent, any further increase in dose resulted in a very slight decrease in surface tension.

The lines of best fit were solved for and equated as outlined previously. The CMC for the soil slurry was determined to be 2.9 % (29,000 mg/l) by this method. Equations of best fit along with their residual values are shown in table 3.3. There were variations in values of surface tension obtained at the same surfactant concentrations for replicate experiments. The most probable explanation for this difference is due to the soil heterogeniety. Soil slurries contained 60 g of soil (dry weight) in order to minimize

heterogeniety but that may have not been sufficient. Another source of error may be due to losse of surfactant due to sorption.

Table 3.3 Linear equations for sub-CMC and supra-CMC regions and their r^2 values for Yorktown soil slurry extracts

Region	Equation of the line for surface tension (dynes/cm)[1]	r^2
Sub-CMC	g = -13.373ln(C) + 170.29	0.743
Supra-CMC	g = -1. (C) + 44.168	0.0225

[1]Note C is in mg/l

Table 3.4 is the surface tension of various Tween 80 surfactant concentrations with and without soil. The table shows the effect that soil has on surface tension with increasing surfactant concentration. Soil lowers the surface tension of water and requires approximately 1% surfactant before surface tension is lowered. Also, the surface tension values determined by best fit equations are also presented for comparison. Regressed surface tension values for DDI water compare favorably to actual values obtained. The regressed values for soil slurries also compare favorably in the supra-CMC region.

Table 3.4. Comparison of Surface Tension of varying Tween 80 concentrations with and without soil and their respective regression outputs

Concentration (mg/l)	w/o soil (dyn/cm)	w/soil (dyn/cm)	w/o soil Regressed	w/soil Regressed
0.00	71.3	59.3		
0.10	71.4		79.9	
0.50	71.3		69.7	
1.00	63.6		65.2	
4.00		60.2	56.4	
5.00	54.0		55.0	
10.0	50.7		50.5	
12.0		59.9	49.4	
15.0	49.2		48.0	
20.0	46.3	60.6	46.1	
25.0	44.2		44.7	
30.0		59.6	43.5	
50.0	43.7	59.9	40.6	
100	40.7	58.8	40.3	
1000	38.7.	55.4	39.3	
5000		51.4	38.6	
7000		51.9	38.4	51.9
10000	38.4	48.3	38.3	47.1
15000		39.8	38.1	41.7
20000		38.1	37.9	37.9
30000		32.9	37.8	32.9
50000		32.3	37.5	32.4
100000	37.3	31.6	37.2	31.6

Summary of experiments conducted to determine CMC in DDI water and soil slurry

The time for equilibration of the Tween 80 surfactant in DDI water is approximately 20 minutes. Results showed that the surface tension decreaseed with increasing surfactant concentration until the critical micelle concentration was reached. The CMC of Tween 80 in DDI water was determined to be 47 mg/l. Beyond the CMC, increased surfactant concentration resulted in little surface tension reduction. This is expected though as reduction in surface tension is due to surfactants in monomeric form and beyond the CMC the surfactant is in micelle form. Surface tension of DDI water was reduced by approximately 32 dynes per centimeter by Tween 80.

Batch soil slurry samples dosed with various concentrations of Tween 80 showed a significant decrease in surface tension simply due to the soil matrix. Both organic matter that leached from the soil and colloidal matter are probably responsible for this reduction in surface tension. Large amounts of the surfactant appeared to sorb to the soil as the surface tension did not decrease until a surfactant concentration of approximately 1%. Figure 5 clearly demonstrates the shift in CMC between DDI water and the soil slurry due to surfactant sorption. The critical micelle concentration was determined to be 2.9 percent with a surface tension of approximately 33 dyne per centimeter.

Explosives Desorption From Yorktown Soil

Desorption Kinetics

This experiment was conducted to determine the equilibrium time of explosives desorption from the Yorktown soil. Figure 3-6 shows the aqueous phase concentration as a function of time. From the plot it is apparent that in the 3% Tween solution equilibrium is reached between 12 and 24 hours. In DDI water, equilibrium is reached between 6 and 12 hours. The surfactant solution is able to achieve an aqueous phase concentration twice that in DDI water. In both systems the total explosives concentration decreases at the final sample interval of 96 hours. A potential explanation for this phenomenon is that the soil structure is sheared by the mixing action and would provide more surface area for sorption. Both surfactant and explosives could sorb to the increased surfaces. An analysis of all explosive compounds showed that there was no significant increase in TNT transformation products which would have been indicative of a reductive process occuring. Additionally, the experiment was designed so that over 90%

Figure 3-7 is the plot of TNTdesorption kinetics. TNT desorption reached equilibrium in DDI water within 12 hours with an aqueous phase concentration of about 80 milligrams per liter. TheTween 80 surfactant solution reached equilibrium in approximately 24 hours. The aqueous phase concentration of TNT in the surfactant solution was considerably higher at nearly 180 milligrams per liter. The aqueous concentration of 4A-DNT in figure 3-8 reached a peak within 12 hours in each system. There was no significant difference in aqueous phase concentrations between DDI water and surfactant solution.. Equilibrium was achieved between 6 and 12 hours for aqueous concentration of 2A-DNT (figure 3-9) in the DDI water system. It appears that equilibrium was never quite achieved for liquid concentration of 2A-DNT in the surfactant system. The 2A-DNT concentration in the surfactant solution was over twice that in DDI water. Figure 3-10 is the plot of RDX desorption kinetics. The RDX desorption was very quick in both systems, with each reaching equilibrium in about 6 hours. No significant difference was noted in aqeous phase RDX concentrations between systems. Figure 3-11 is the plot of HMX desorption kinetics. HMX took 24 hours to reach equilibrium in the DDI system and between 24 and 48 hours in the surfactant

enhanced system. The HMX concentration in the surfactant enhanced system was also nearly double of that in the DDI water system.

From the kinetic desorption experiments it was evident that a state of quasi-equilibrium for explosive compounds was reached within 24 hours. This quasi-equilibrium time was used as the time interval for the batch desorption experiments.

Batch Desorption

Batch desorption experiments were conducted to determine the efficacy of three different nonionic surfactants for desorbing explosive compounds from soil. The three nonionic surfactants analyzed were Tween 80, Witconol SN 120, and Simple Green. After the 24 hour shake period, soil slurry samples were centrifuged, and the liquid and solid phases were separated. The aqeous phase was filtered and both soil and aqueous portions were analyzed for explosive compounds by EPA Method 8330 with a reverse phase high performance liquid chromatograph (HPLC).

Aqueous phase and soil phase concentrations are tabulated in Appendix C for each surfactant studied. Total explosives were determined by the sum of the soil and aqueous phase concentrations for each sample. There is no conclusion that can be drawn from a comparison of the aqueous phase concentration on a component by component basis for all surfactants tested. This is due to the heterogeniety of the soil. Each sample had a different concentration of explosive compounds since the contaminant distribution was not uniform. Thus, in order to analyze the data, it had to first be normalized on a percentile basis. The percent desorbed of each contaminant was determined by the mass in the aqueous phase divided by the the total original mass on the solid phase.

This calculation was repeated for each sample. Plots of percent desorbed for each contaminant (total explosives, TNT, sum Amino-DNT, RDX, and HMX) versus surfactant concentration for all three nonionic surfactants are provided (Figures 3-13 - 3-16). In general, all contaminants show the same general desorption trend with the only exception being TNT.

Figure 3-13 is the plot for total explosives. This plot shows a slight initial concentration decrease followed by a dramatic increase in percent desorbed as the dose approaches 3% for both Witconol SN 120 and Tween 80. For the Tween 80 and Witconol runs, the control of DDI water (zero percent surfactant sample) desorbed 41 and 43 percent respectively. At a dose of 3 percent, Tween 80 desorbed 65 percent while Witconol managed a slightly higher 68 percent desorption of total explosives. Additional amounts of surfactant added beyond 3 percent had much less of an impact upon percent desorbed. Simple Green appears to have achieved the same percent desorbed as DDI water throughout all surfactant concentrations examined.

Similar results for each compound (TNT, sum Amino-DNT, RDX, and HMX) are shown in figures 3-14 - 3-16. Similiar to the plot for total explosives, each compound should an increase of percent desorbed as surfactant concentration increased for Tween 80 and Witconol SN 120 with Simple Green showing little effect. The decrease in percent desorbed for contaminants between surfactant concentrations less than 1% is due to surfactant monomers sorbing to the soil. The monomer will coat the soil and the contaminant in the aqueous phase will partition with the monomer to the soil. Thus, until CMC is reached (3% for Tween 80) it is not suprising at low surfactant concentrations to have lower percent desorbed than DDI water. It appears that the trend line level of indicating that the contaminant reached a maximum percent desorbed at approximately 3% surfactant concentration. The use of a comparison of means statistical measure

(Fisher's Least Protected Significant Difference) showed that percent desorbed was significant at 3% surfactant concentration.

From reviewing figures 3-13 - 3-16 and the data tables in Appendix C, it is apparent that Tween 80 and Wiconol SN 120 desorbed significant amounts of contaminants at 3% concentration while Simple Green did not.

Sequential Batch Desorption

The sequential batch desorption experiments were conducted to sequentially challenge the soil with DDI water and surfactant solutions to examine desorption over time. This experiment also allowed a comparison between both Tween 80 and Witconol SN 120 at doses of three percent and five percent. The results are also used to develop desorption isotherms. Desorption isotherms generate information about desorption with respect to initial explosives concentrations and the amount of total explosives that can be desorbed from the soil.

Explosive compound concentrations for each sample interval are provided in Appendix D. The concentration of each compound was determined from the final soil concentration and adding the aqueous phase concentration for each day to determine the total explosives for each day. To convert the aqueous phase concentration to soil concentration, the aqueous phase concentration was multiplied by the liquid volume and divided by the dry soil weight of the sample.

Once daily soil component concentrations were calculated, the triplicate sample concentrations for both soil and water were averaged for each day. Once the average values were determined, plots of equilibrium concentration versus soil concentration were used to develop desorption isotherms for each case. Linear, Freundlich, and Langmuir models were plotted using the data to determine which was the best fit.

The Langmuir model did not fit any of the data as the plot of the inverse of equilibrium concentration versus the inverse of soil concentration (equation 9) was not linear. However, both the linear model and the Freundlich model did fit the data. The linear model was the best fit overall. Linear and Freundlich models were fit for each explosive component in each case with only the following exceptions. Desorption of HMX in pure water, HMX in Tween 80 solution, and TNT in the three percent Witconol solution could not be modeled. Every other case could be fit with both models with the square of the sample correlation coefficient remaining above 75 percent. Tables 3-5 through 3- 9 list regression derived formulas for each case along with their respective correlation coefficients. In all cases, the linear model was the best fit overall. Desorption isotherms are provided in Figures 3-17 - 3-28.

A review of the linear isotherms provided in Figures 3-17 - 3-28 show some general trends. The benefit of adding surfactants (both Tween 80 and Witconol SN 120) is readily demonstrated as the aqueous phase explosives concentration is always greater than DDI water irregardless of the soil concentration. In fact, at very low soil concentrations (< 50 mg/kg) the surfactants are able to desorb explosives while the DDI water cannot. This result is interesting in that as the explosive compounds are degraded with time in the bioreactor, it appears that desorption will cease in a reactor that does not utilize surfactant. A system with surfactants continues to show explosives desorption so that theoretically biotreatment of the soil should be more complete. Additionally, it does not appear that there is a significant difference between surfactants at 3 and 5% concentrations as their isotherms are very similiar. The benefit gained from an additional 20,000 mg/l of surfactant is minimal. Finally, it appears that the desorption of explosives is mass transfer limited. The sterilization of the soil (via autoclave) resulted in the transformation of TNT and the other explosive compounds. The net result are a decrease in concentration of explosive compounds

much below that typically found. This decrease in explosives concentration resulted in the solubility limit of the compounds not being reached during desorption and therefore, mass transfer became controlling.

The distribution coefficient, K_F', in the Freundlich model is a an indication of a contaminant partitioning between phases. The number is a ratio of soil to water and by its relative magnitude indicates the phase it will tend to favor. Relatively speaking, a high number indicates soil partitioning and a low number indicates aqueous partitioning.

Examining the total explosives component, the K_F for DDI water is equal to 201. In pure water, the value of the distribution coefficient for TNT was 19.5 while the value for RDX was 45.7. Values for 4A-DNT and 2A-DNT were 100 and 75.8 respectively in pure water. Poor results were obtained for isotherm regression when analyzing HMX, for which case the plot was more vertical in a sense. This vertical case is an indication of a solubility limitation rather than a desorption limited case [Pennington et al, 1995].

Table 3-5. Explosive component isotherm equations for DDI water and Yorktown soil

	Equation (S_i =)		r^2	
Component	Linear	Freundlich	Linear	Freundlich
Total Explosives	$1.57x + 249$	$201x^{0.135}$	0.989	0.918
TNT	$0.798x + 17.4$	$19.5x^{0.0684}$	0.995	0.880
RDX	$0.733x + 43.7$	$45.7x^{0.113}$	0.999	0.793
HMX	N/A	N/A	N/A	N/A
4A-DNT	$1.99x + 104$	$100x^{0.118}$	0.999	0.918
2A-DNT	$2.31x + 72.7$	$75.8x^{0.074}$	0.999	0.915

Adding three percent Tween 80 to the sequential batch solutions greatly reduced the distribution coefficient values and therfore increased the solubility of the contaminant. The coefficient for total explosives was reduced from 201 to a value of 43.4. A value of 45.7 for the distribution coefficient of RDX in water was decreased to 6.36 in three percent Tween 80. The coefficient for 4A-DNT was decreased nearly four times to a value of 28.9 while the coefficient for 2A-DNT was almost halved to a value of 37.8. The desorption of TNT was the only exception to this trend with a slight rise in the distribution coefficient from 19.5 for water to 23.6 for 3 percent Tween 80.

Table 3-6. Explosive component isotherm equations for 3% Tween 80 water and Yorktown soil

	Equation (S_i =)		r^2	
Component	Linear	Freundlich	Linear	Freundlich
Total Explosives	$3.28x + 139$	$43.4x^{0.520}$	0.997	0.965
TNT	$1.89x + 27.8$	$23.64x^{0.377}$	0.987	0.890
RDX	$3.64x - 4.35$	$6.36x^{0.788}$	0.939	0.908
HMX	N/A	N/A	N/A	N/A
4A-DNT	$2.88x + 50.3$	$28.9x^{0.466}$	0.993	0.977
2A-DNT	$4.00x + 42.1$	$37.8x^{0.340}$	0.995	0.981

When 5 percent Tween 80 (Table 3-7) was used to challenge the Yorktown soil, slightly lower values for desorption distribution coefficients were achieved below those for 3 percent Tween. One exception was noted however, the value for the distribution coefficient for RDX slightly increased in the 5 percent surfactant as compared to the 3 percent Tween 80. The most remarkable statistic which can be found from this analysis of the 5 percent isotherms is that the distribution coefficient for TNT was decreased to nearly half of that for pure water.

Table 3-7. Explosive component isotherm equations for 5% Tween 80 and Yorktown soil

	Equation (S_i =)		R^2	
Component	Linear	Freundlich	Linear	Freundlich
Total Explosives	$2.77x + 87.2$	$33.1x^{0.517}$	0.984	0.948
TNT	$1.96x + 15.7$	$10.2x^{0.590}$	0.979	0.907
RDX	$2.98x + 0.562$	$6.96x^{0.699}$	0.912	0.901
HMX	N/A	N/A	N/A	N/A
4A-DNT	$2.36x + 27.6$	$20.2x^{0.458}$	0.999	0.964
2A-DNT	$3.22x + 28.2$	$29.0x^{0.295}$	0.996	0.979

Reduction in distribution coefficients when Witconol SN 120 was applied were significant, however they were not as great as reductions achieved by Tween 80. The value of the distribution coefficient as applied to total explosives was twice that found for three percent Tween 80. The coefficient for three percent Witconol was 88.4 as compared to 43.4 for three percent Tween 80. However, Witconol SN 120 did appear to perform better than Tween 80 in respect to the desorption of the TNT breakdown products 2A and 4A-DNT.

Table 3-8. Explosive component isotherm equations for 3% Witconol and Yorktown soil

	Equation (S_i =)		r^2	
Component	Linear	Freundlich	Linear	Freundlich
Total Explosives	$1.13x + 117$	$88.4x^{0.213}$	0.984	0.800
TNT	N/A	N/A	N/A	N/A
RDX	$0.753x + 7.78$	$9.33x^{0.358}$	0.990	0.790
HMX	$2.78x + 12.0$	$17.1x^{0.346}$	0.975	0.752
4A-DNT	$1.30x + 26.6$	$25.7x^{0.287}$	0.996	0.813
2A-DNT	$1.41x + 20.8$	$24.0x^{0.169}$	0.991	0.790

An increase in surfactant concentration of Witconol SN 120 from three percent to five percent showed significant decreases in distribution coefficients. The coefficient for total explosives was nearly cut in half as compared to three percent value. In addition the coefficient for RDX was cut by almost a third.

Table 3-9. Explosive component isotherm equations for 5% Witconol and Yorktown soil				
	Equation (S_i =)		r^2	
Component	Linear	Freundlich	Linear	Freundlich
Total Explosives	$1.04x + 94.2$	$45.3x^{0.345}$	0.979	0.975
TNT	N/A	N/A	N/A	N/A
RDX	$0.781x + 8.20$	$6.99x^{0.485}$	0.995	0.921
HMX	$2.39x + 23.8$	$23.7x^{0.323}$	0.973	0.873
4A-DNT	$0.981x + 20.8$	$20.8x^{0.277}$	0.999	0.843
2A-DNT	$1.36x + 17.5$	$18.1x^{0.255}$	0.966	0.941

Inspection of figures 3-7 and 3-8 is the easiest way in which to understand the effect that the addition of the surfactants to the soil slurry has on the desorption of total explosives. For each isotherm, the point with the greatest aqueous phase concentration represents data corresponding to day one of the sequential batch desorption. The point with the least aqueous concentration represents the final day. Initial total explosives for the surfactant solutions were considerably higher than those for water alone. Initial explosives concentrations for Tween 80 with doses of three percent and five percent were on the average from 140 to 160 milligrams per liter. Total concentration in pure water were only around 90 milligrams per liter. Witconol was even more effective initially, pulling off nearly 180 milligrams per liter.

Each subsequent washing resulted in higher aqueous concentrations of total explosives in the surfactant solutions than in those for water alone. Finally, the final soil concentrations for total explosives after the fourth day of washing are much lower for surfactant enhanced solutions than those for DDI water. With water alone, around 250 milligrams of total explosives per kilogram of soil remained. With the addition of three percent Tween 80 that value was decreased to just below 200 milligrams per kilogram and to almost 100 milligrams per kilogram for 5 percent Tween 80. Both concentrations of Witconol managed to decrease the total concentration of explosives to about 100 milligrams per kilogram in this example.

Initial concentrations of TNT were much higher with the surfactant enhanced solutions. While pure water was only able to desorb slightly more than seven milligrams of TNT per liter of solution, Witconol solutions were able to desorb from 13 to 15 milligrams per liter. Initial concentrations of TNT in Tween 80 solutions were far greater, ranging from near 50 to almost 55 milligrams per liter. However, it could not be demonstrated that the surfactant was any more efficient at reducing the final soil concentration below that achieved by water. In fact, water had a slightly lower final soil concentration on the fourth day as compared to both surfactants which may be due to great differences in initial TNT concentrations on the soil. Surfactant solutions apparently had as much as 5 to 6 times the initial concentration of TNT as compared to pure water soil samples. However, all initial aqueous concentrations did not approach the reported solubility of TNT in water which is about 100 milligrams per liter according to the Merck index.

The reported solubility for HMX in water is 5 milligrams per liter. Desorption isotherms for any type were not good fits for HMX in water as well as Tween 80. Poor fit may have been caused by solubility limitations in this case. Aqueous concentrations for pure water on days one and two were near the reported solubility limit. Likewise, Tween samples for these same days were at or slightly above the solubility limit. First day concentrations for HMX in Tween slurry aqueous extracts were as high as 7 to 8 milligrams per liter. Witconol was very successful in solubilizing HMX with initial values in solution as high as 18 milligrams per liter. It seems that Witconol was so successful in solubilizing HMX that it was possible to model its desorption. In other words the process was not solubility driven in the case of Witconol but

rather it was desorption driven. Final soil concentrations were not significantly lower with the use of surfactant with respect to HMX. Again, similar to TNT, initial HMX soil concentrations were also higher in the surfactant enhanced samples.

The initial aqueous sample when analyzed for RDX was 35 to 42 milligrams per liter for Tween solutions while for water they were slightly higher averaging almost 50 milligrams per liter. Witconol solutions achieved a higher initial aqueous concentration of RDX than that of water achieving an average value around 75 milligrams per liter. The reported solubility of RDX in water ranges from 42.6 milligrams per liter at 20 °C to about 60 milligrams per liter at 25 °C. Both surfactants were much more successful at desorbing RDX from the soil with Tween and Witconol lowering the soil concentrations below 20 and 10 milligrams per kilogram respectively. The final soil concentration of RDX in the water only samples averaged almost 45 milligrams per kilogram. Therefore the surfactants increased long term desorption by 2.5 to 4.5 times that of water.

Surfactant enhanced solutions appear to have much success in desorbing the TNT breakdown products of 2A and 4A-DNT. With both surfactants at both concentrations the initial aqueous concentrations were higher and the final soil concentrations were lower than that of pure water. The distilled deionized samples averaged initial aqueous concentrations of 20 milligrams per liter for 4A-DNT and between 7 and 8 milligrams per liter for 2A-DNT. Average initial aqueous values for Tween 80 ranged from about 37 to 43 milligrams per liter 4A-DNT while those for 2A-DNT were between 9 and 12 milligrams per liter. Witconol performed the best of all with respect to both breakdown products. Initial aqueous concentrations of 4A and 2A-DNT for Witconol samples average 60 and about 15 milligrams per liter respectively. Final soil concentrations of the breakdown products were 105 milligrams per kilogram for 4A-DNT and 73 milligrams per kilogram for 2A-DNT in the water/soil system. Final average soil concentrations for Tween 80 ranged from 30 to 60 milligrams per kilogram for 4A-DNT and 30 to 50 milligrams per kilogram for 2A-DNT. Witconol averaged about 25 milligrams per kilogram as its final concentration for 4A-DNT. Values for 2A-DNT averaged near 20 milligrams per kilogram with Witconol enhancement.

Summary of kintic, batch and sequential batch desorption experiments

Overall the surfactants Tween 80 and Witconol SN 120 were significantly more effective in desorbing explosives from the Yorktown soil than was pure water alone. Simple green was not effective in enhancing desorption of the explosive components. Experiments to determine the CMC for Tween 80 showed that 3% w/w was the optimal concentration for a soil slurry. Kinetic desorption studies demonstrated that equilibrium was reached rather quickly and equilibrium was within 24 hours. Batch desorption studies show that both surfactants Tween 80 and Witconol were significantly more effective than water in increasing the percent desorbed of total explosives and that the optimal surfactant concentration was 3%. Sequential desorption studies demonstrated that successive applications of surfactant enhanced desorption of explosives compared against DDI water. Sequential batch studies also showed that not only could more initial explosives be desorbed from the soil but that more would ultimately be removed from the soil than DDI water could achieve. It is difficult to conclude which surfactant, Tween 80 or Witconol, is more effective at desorbing explosives from this soil. Both surfactants appear relatively equal in their effectiveness to desorb explosives. Thus, based on similiar results between Tween 80 and Witconol SN 120, we decided to use Tween 80 for bench scale biological remediation experiments due to our past operational experience with that surfactant.

Conclusions

- The critical micelle concentration (CMC) of Tween 80 in DDI water was determined to be 47 mg/l.
- The CMC of Tween 80 in a 30% (w/w) soil slurry was determined to be 3%.
- Simple Green surfactant did not enhance the desorption of explosive compounds from Yorktown soil.
- Witconol SN 120 and Tween 80 performed comparably; and both were significantly better than DDI water in enhancing explosives desorption.
- The optimal dose of Witconol SN 120 and Tween 80 was determined to be 3%.
- Sequential batch desorption data of explosive compounds fit both Freundlich and linear models but the linear model had the best fit.
- The linear isotherms showed the benefit of added surfactant over DDI water in the desorption of explosives over time. Additionally, the isotherms demonstrated the surfactants' ability to enhance desorption of contaminants at very low soil concentrations.
- The surfactant Tween 80 was chosen for use in the surfactant enhanced treatment process to be used in bench scale biological remediation experiments of explosive contaminated soils from Yorktown, Virginia.

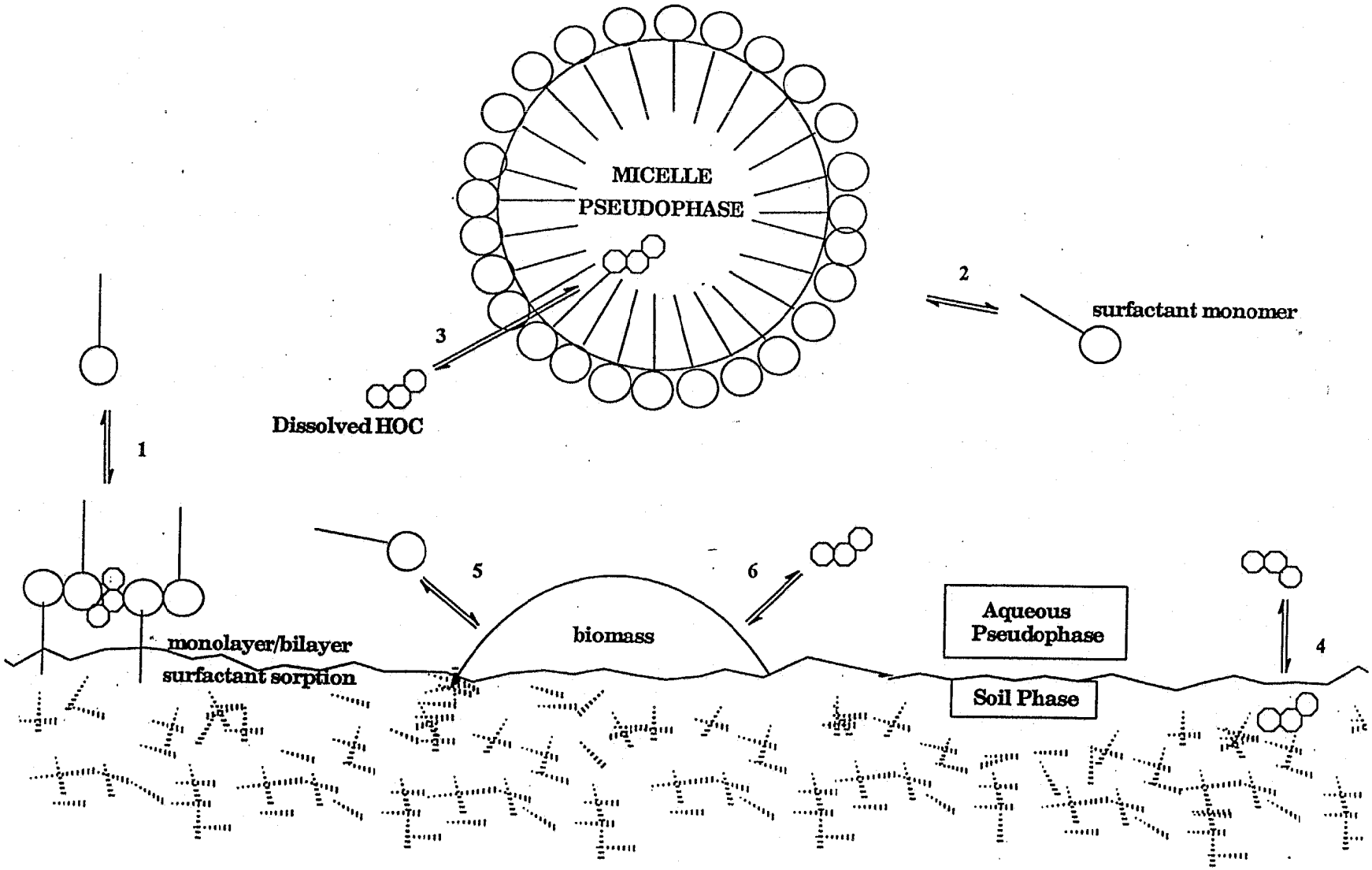

Figure 1-2. Conceptual model of coupled HOC sorption, micellar solubilization and biodegradation (Pennell and Abriola 1996).

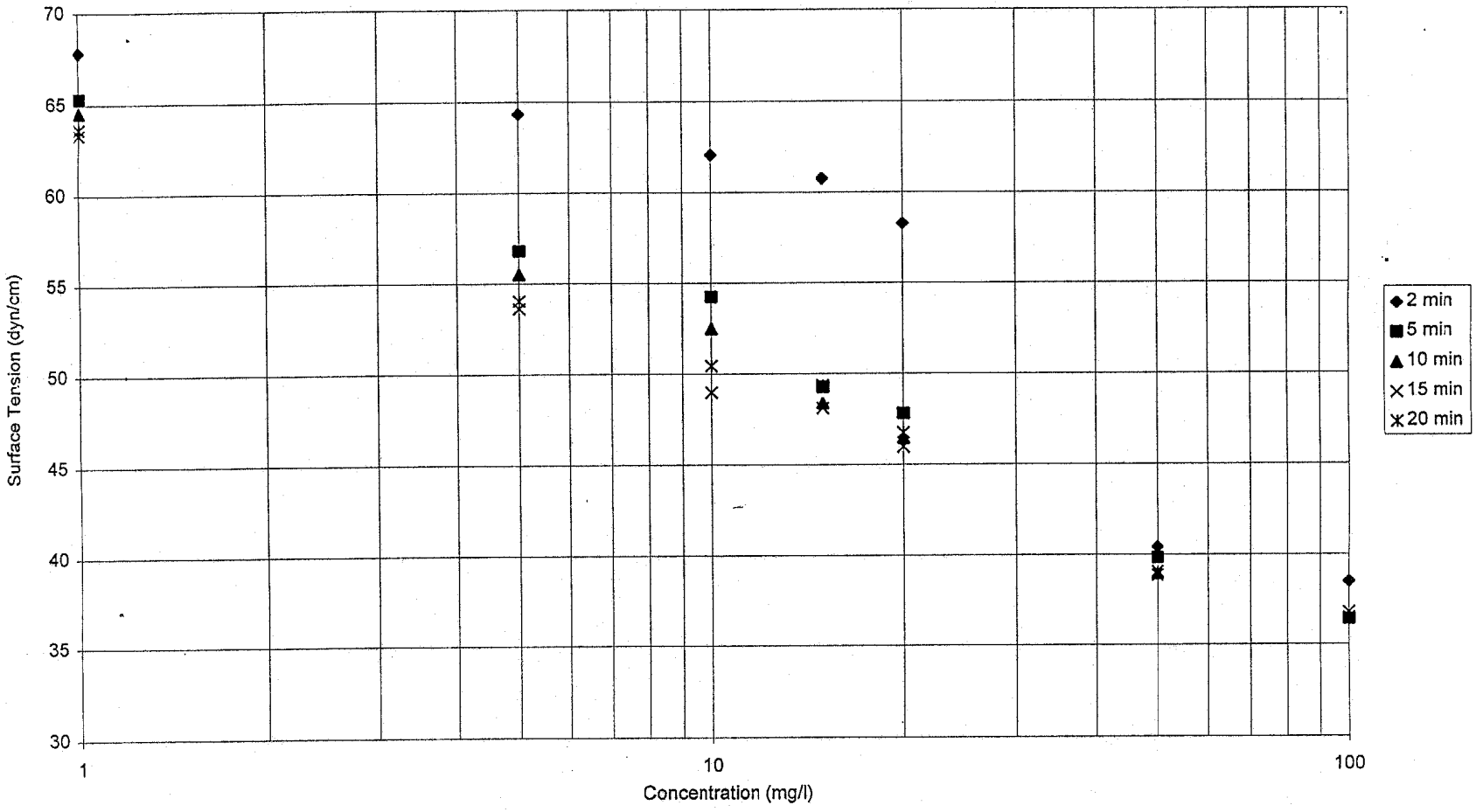

Figure 3-1. Plot of surface tension versus surfactant concentration at various time intervals for a surfactant solution of Tween 80 in DDI water.

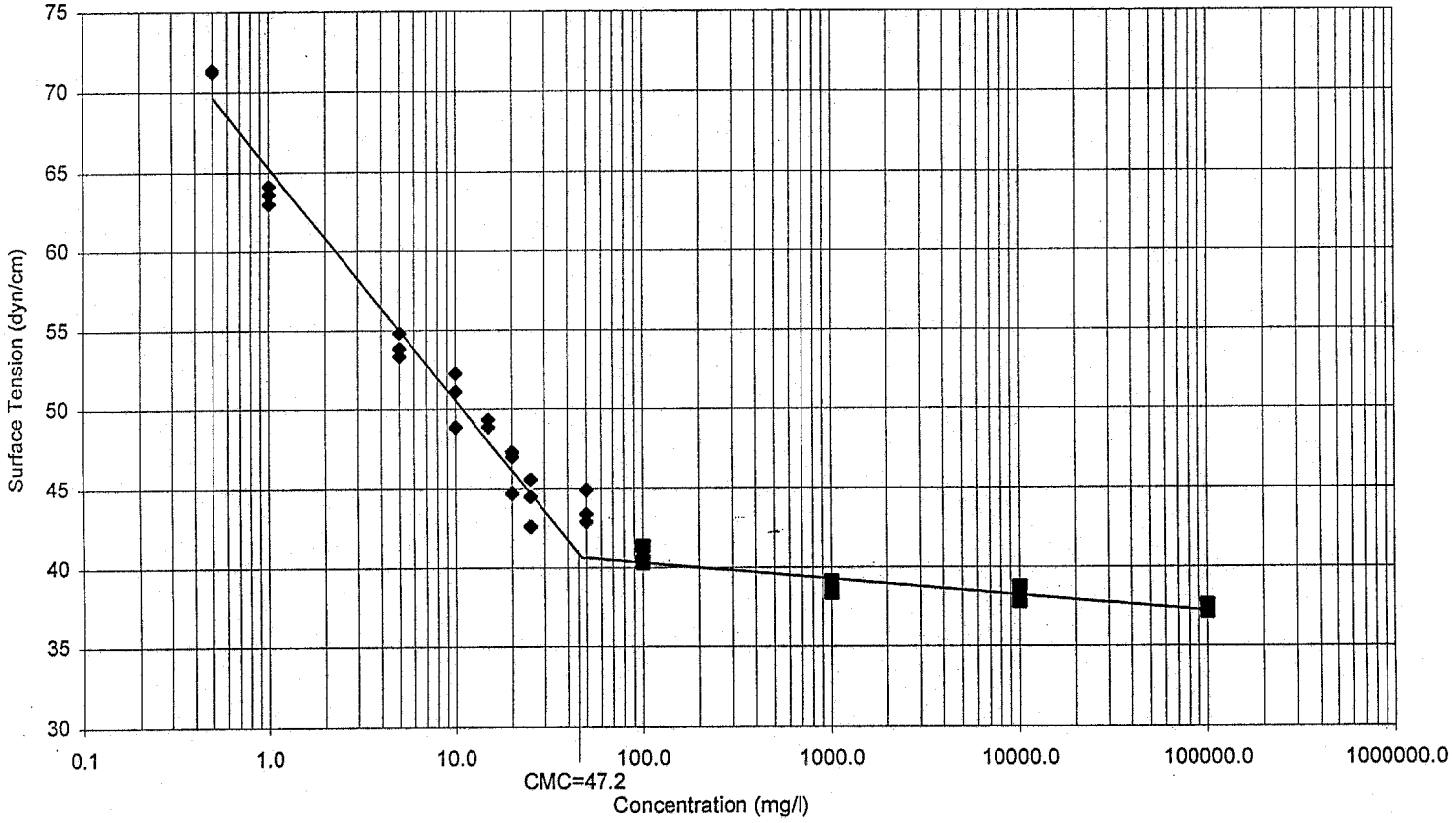

Figure 3-2. Graphical determination of the CMC in a solution of Tween 80 and DDI water.

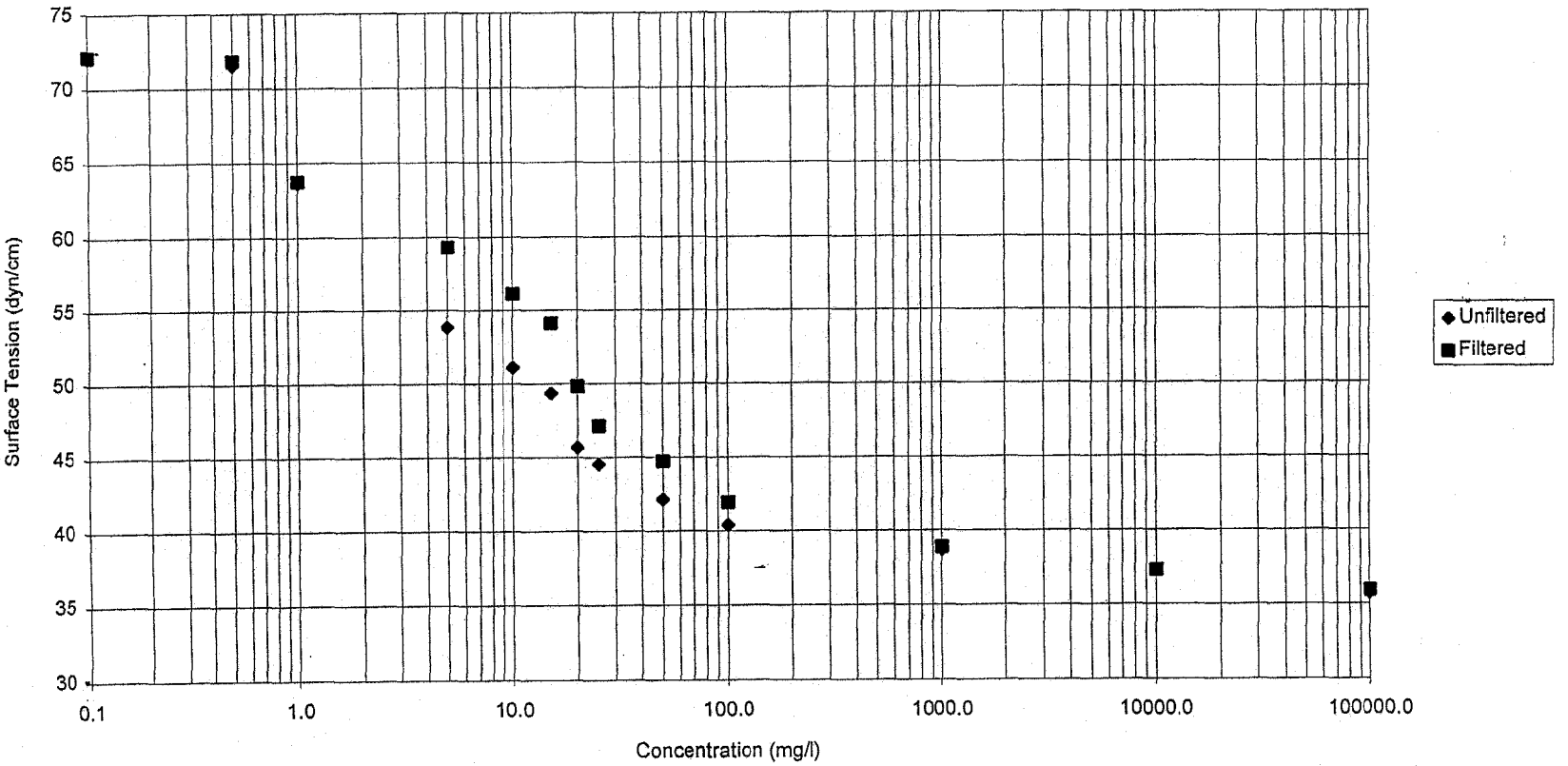

Figure 3-3. Plot of surface tension versus surfactant concentration of Tween 80 in water demonstrating the CMC shift due to surfactant loss.

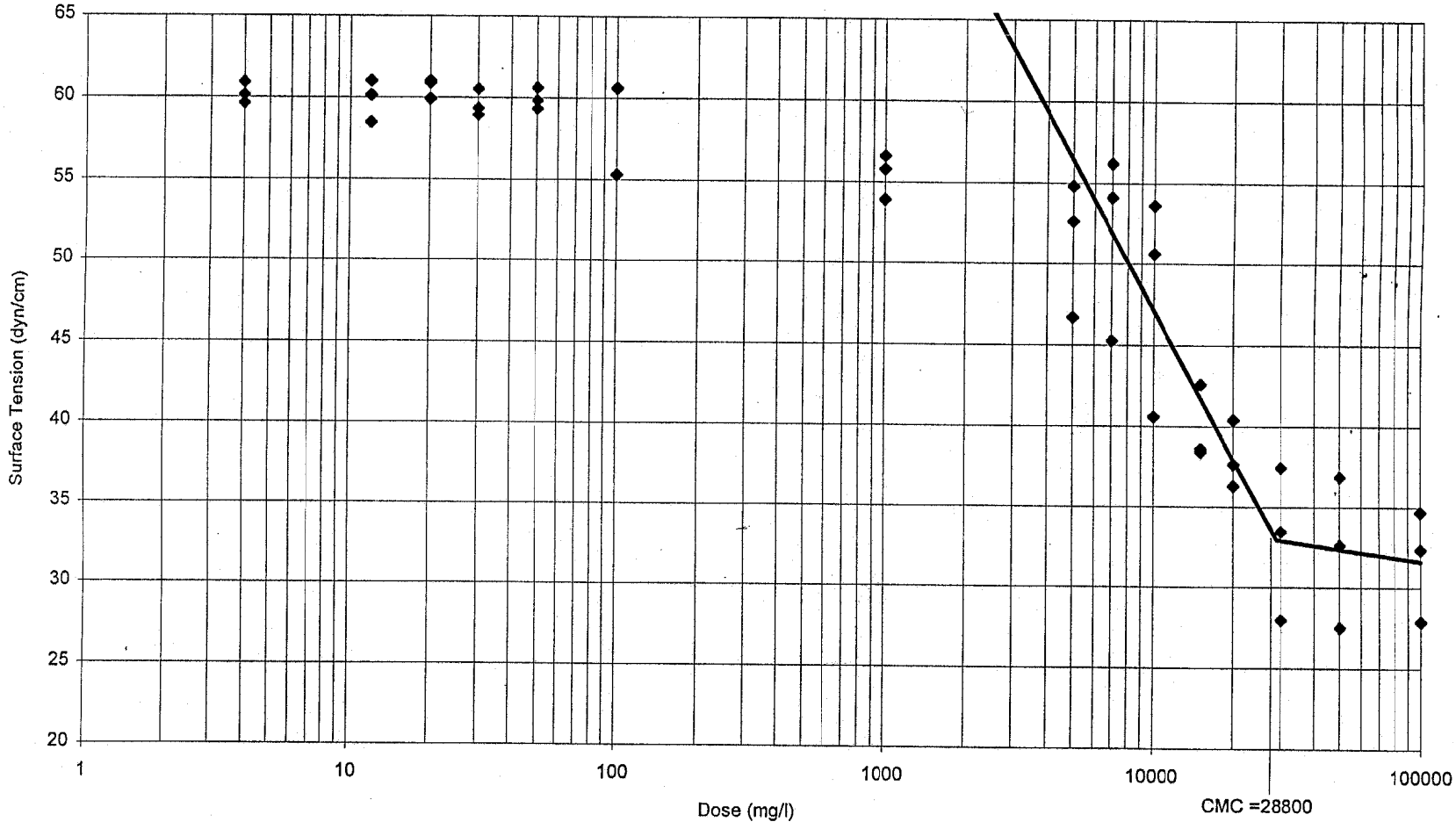

Figure 3-4. Plot of surface tension versus Tween 80 surfactant concentration to determine the CMC in soil slurries.

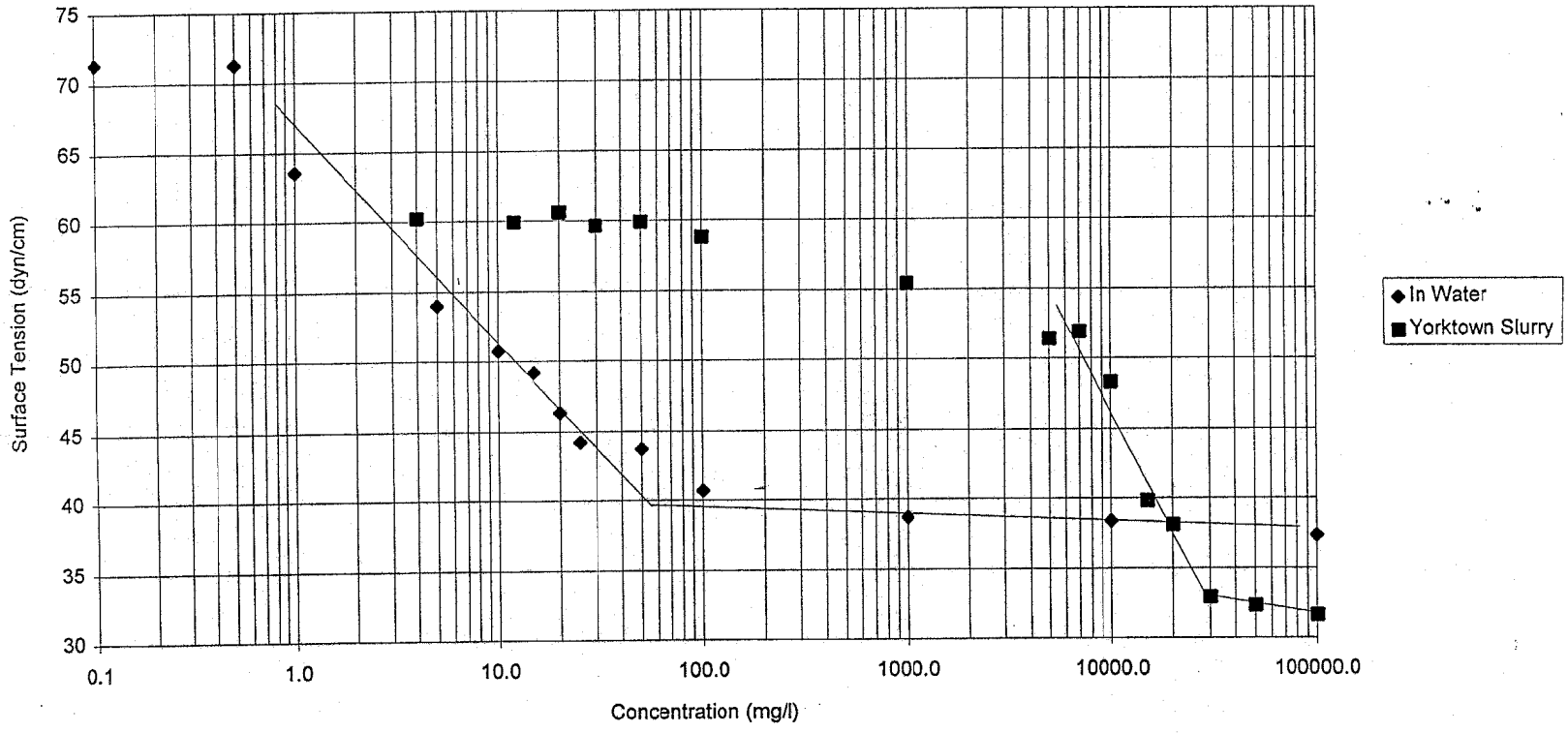

Figure 3-5. Plot of surface tension versus surfactant concentration for DDI water and soil slurry. The plot demonstrates the shift in the CMC between DDI water and soil slurry due to sorption of surfactant on soil.

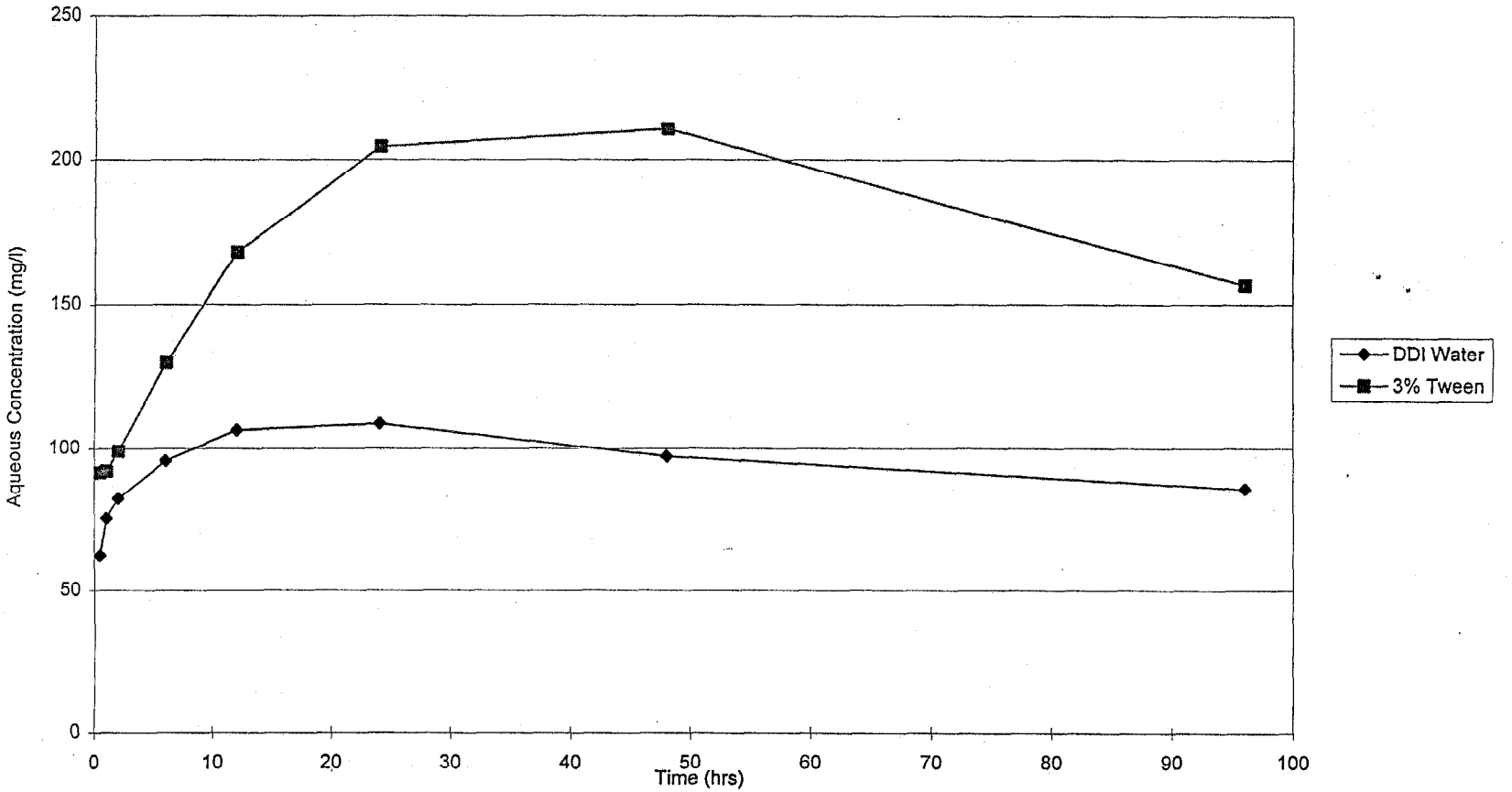

Figure 3-6. Desorption kinetics for total explosives from Yorktown soil in DDI water and 3% Tween 80.

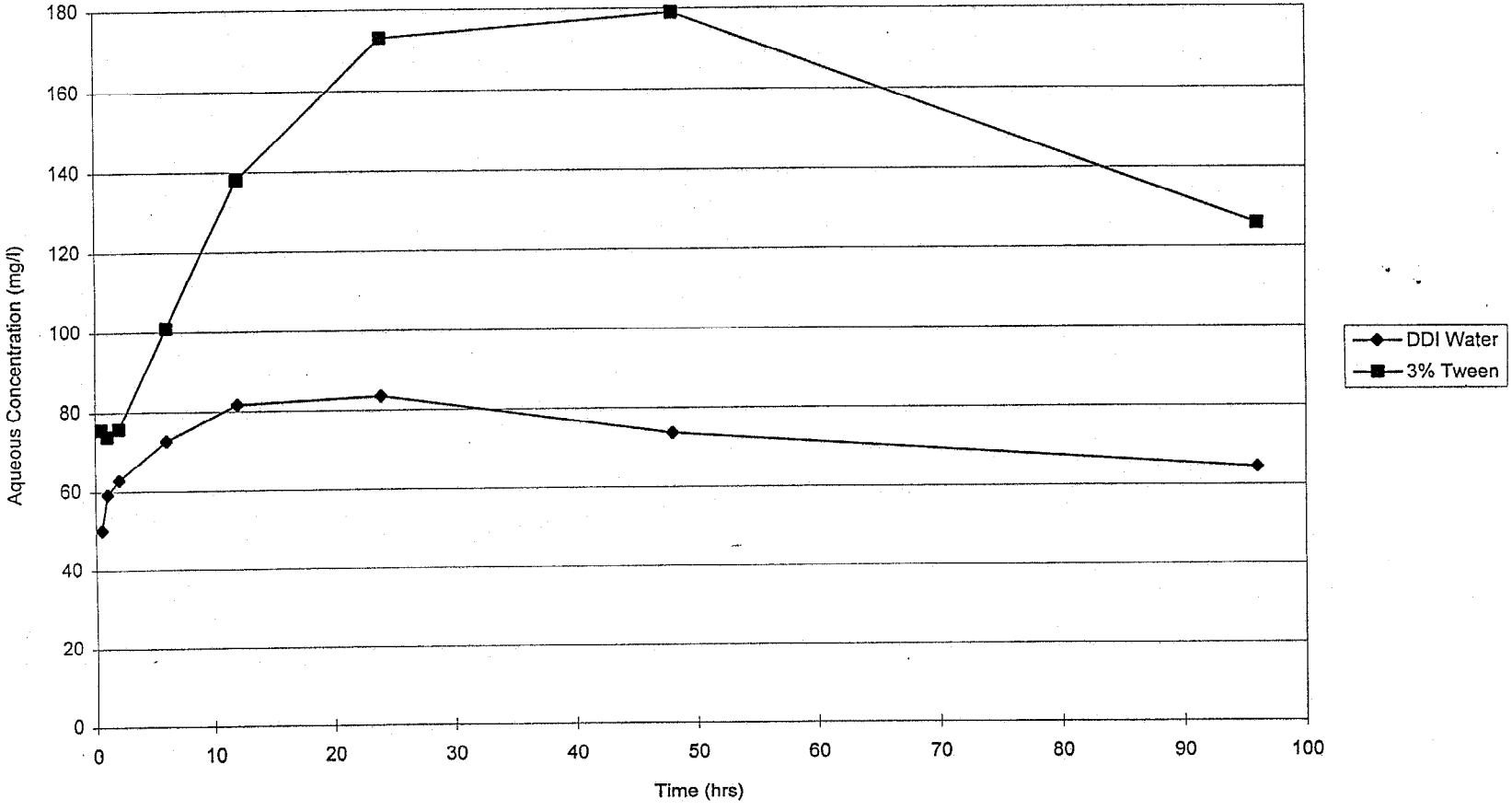

Figure 3-7. Desorption kinetics for TNT from Yorktown soil in DDI water and 3% Tween 80.

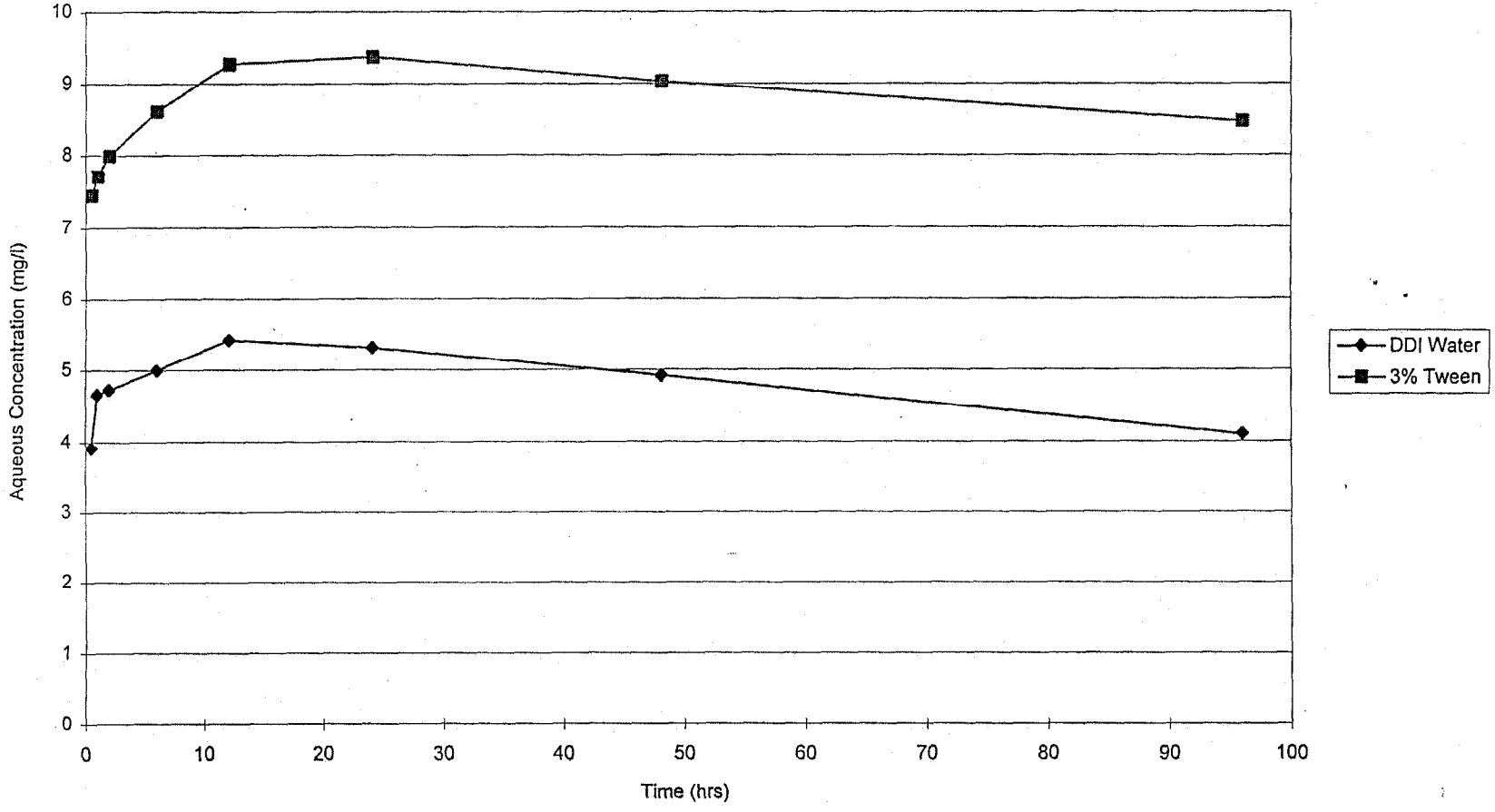

Figure 3-8. Desorption kinetics for 4A-DNT from Yorktown soil in DDI water and 3% Tween 80.

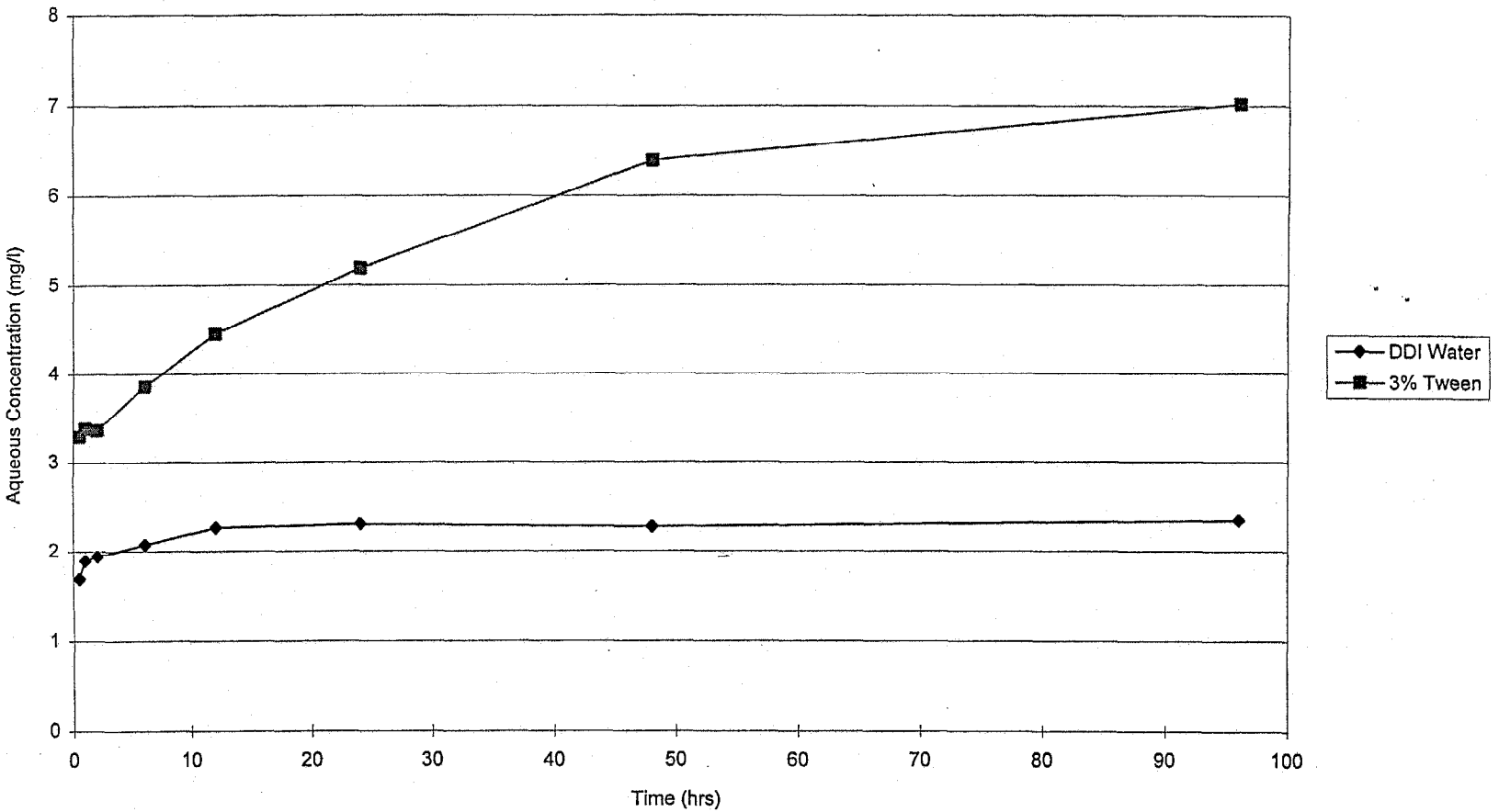

Figure 3-9. Desorption kinetics for 2A-DNT from Yorktown soil in DDI water and 3% Tween 80

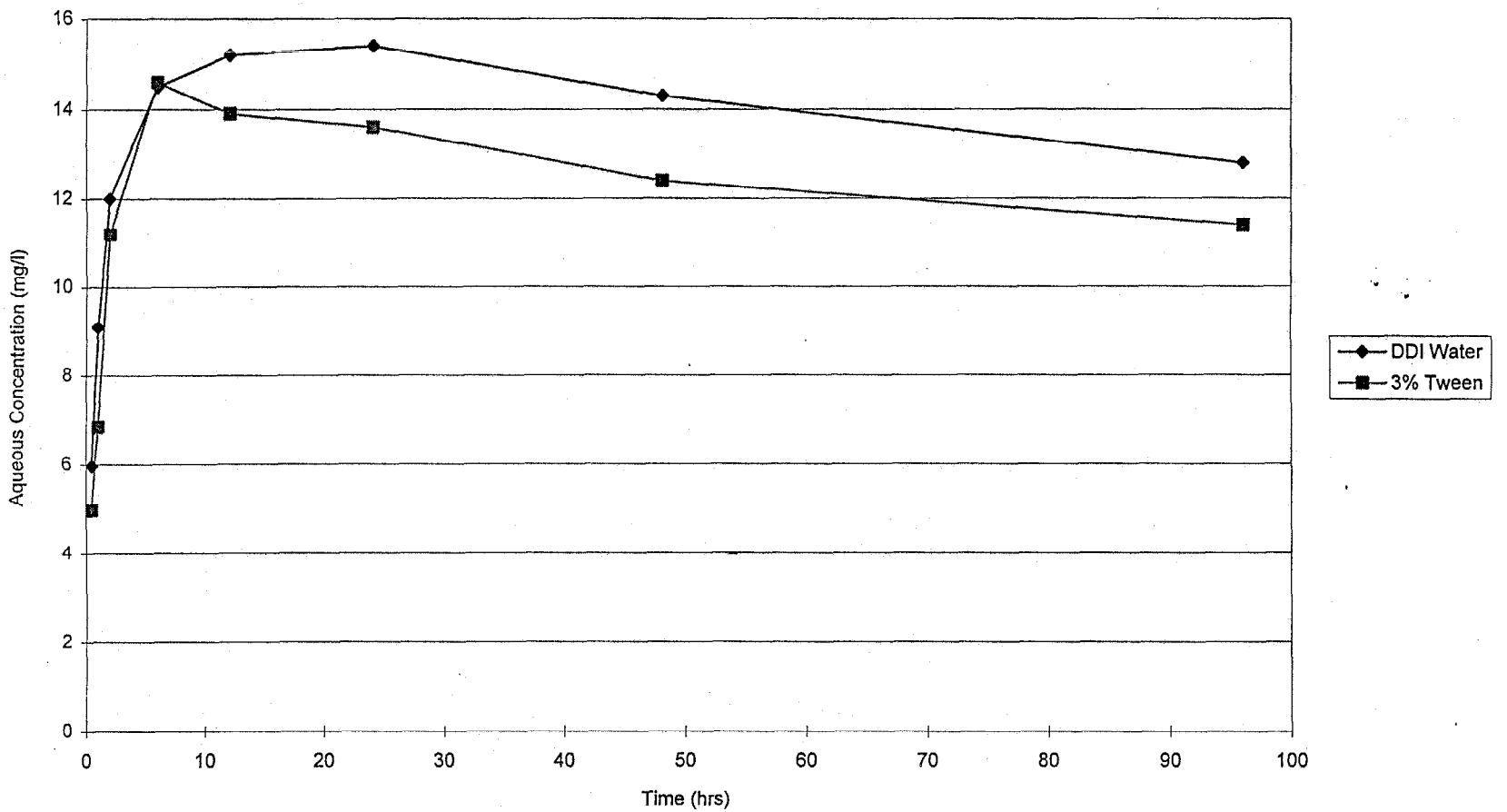

Figure 3-10. Desorption kinetics for RDX from Yorktown soil in DDI water and 3% Tween 80.

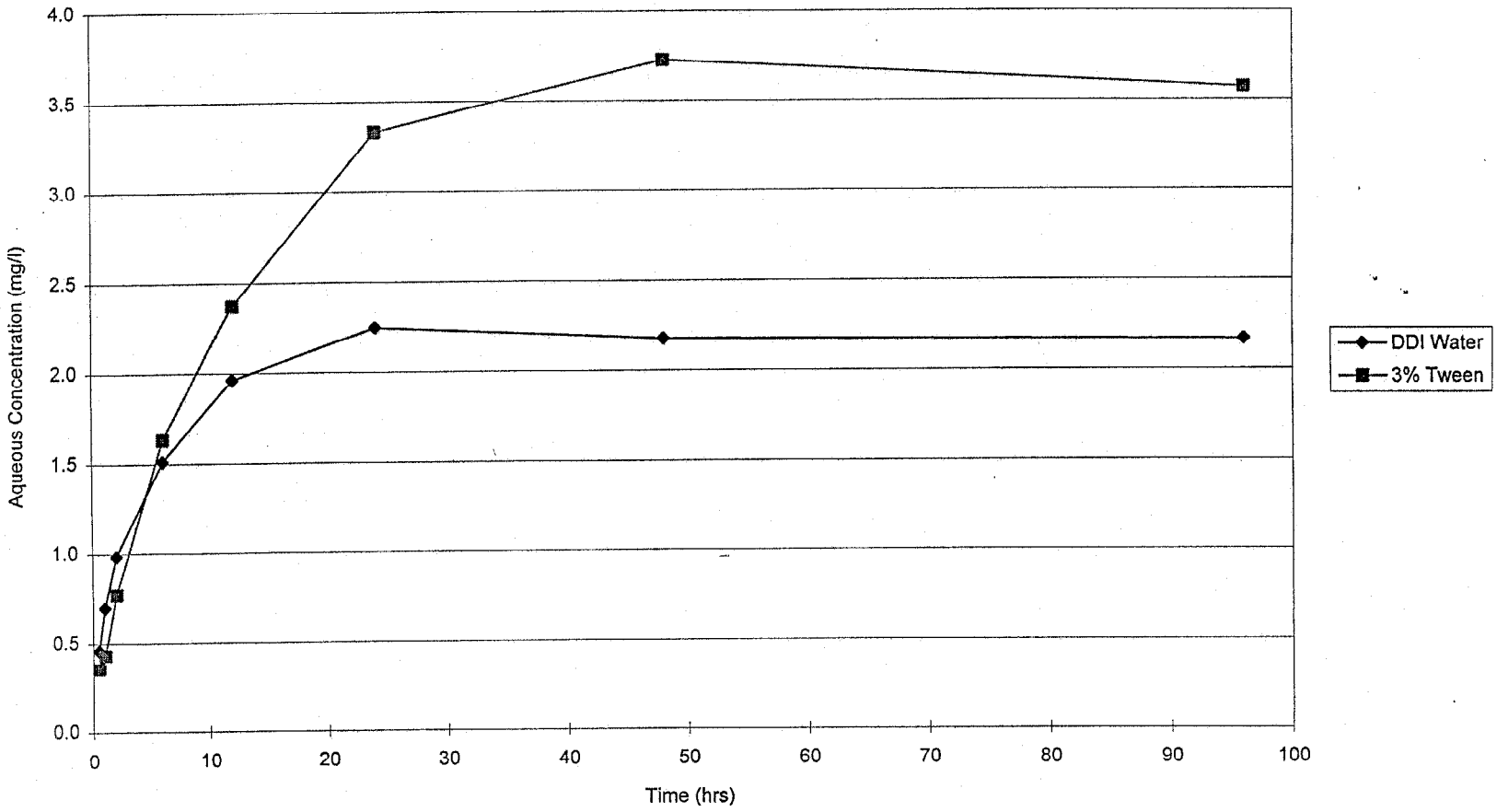

Figure 3-11. Desorption kinetics for HMX from Yorktown soil in DDI water and 3% Tween 80.

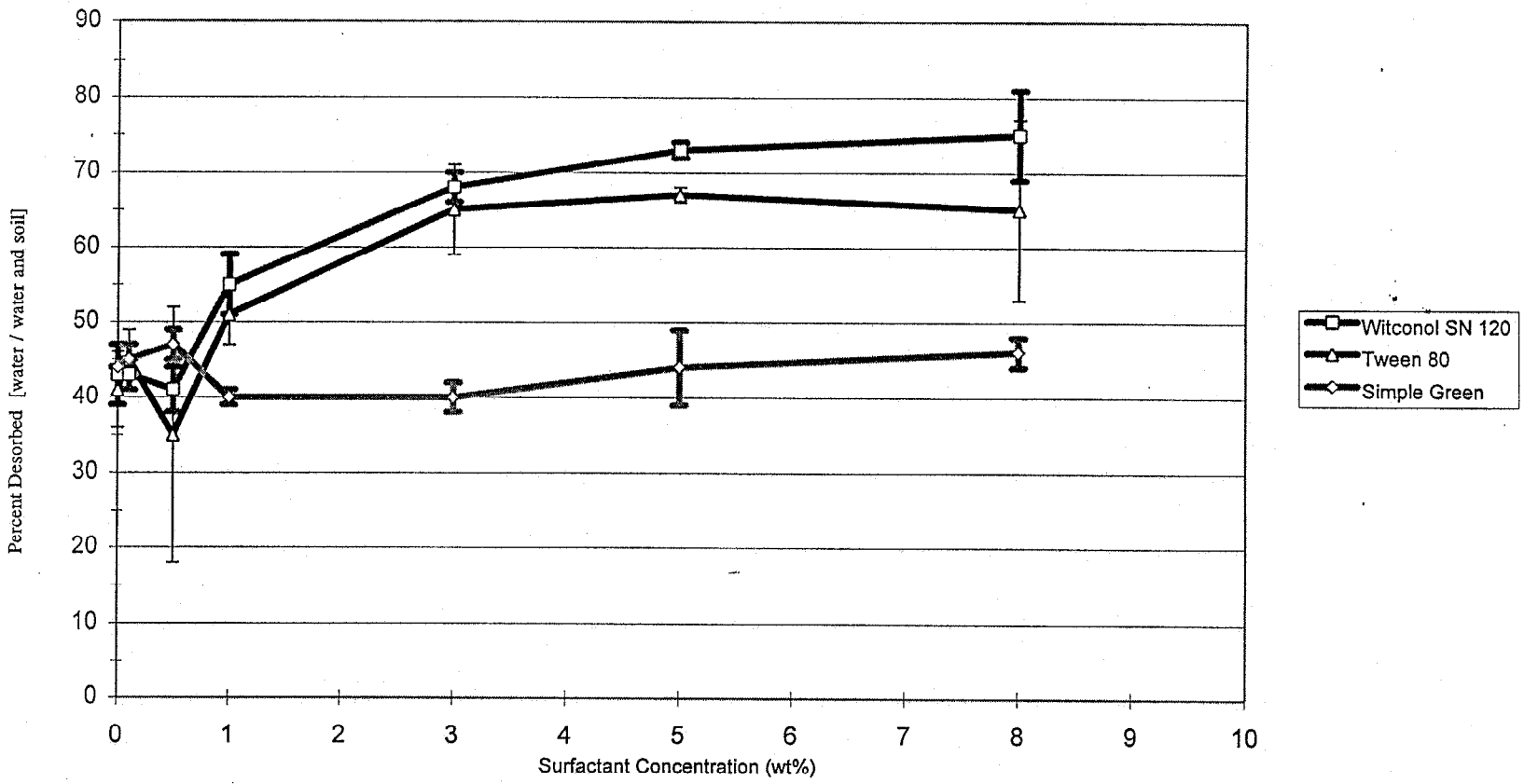

Figure 3-12. Comparison of three nonionic surfactants in the batch desorption of total explosives from soil.

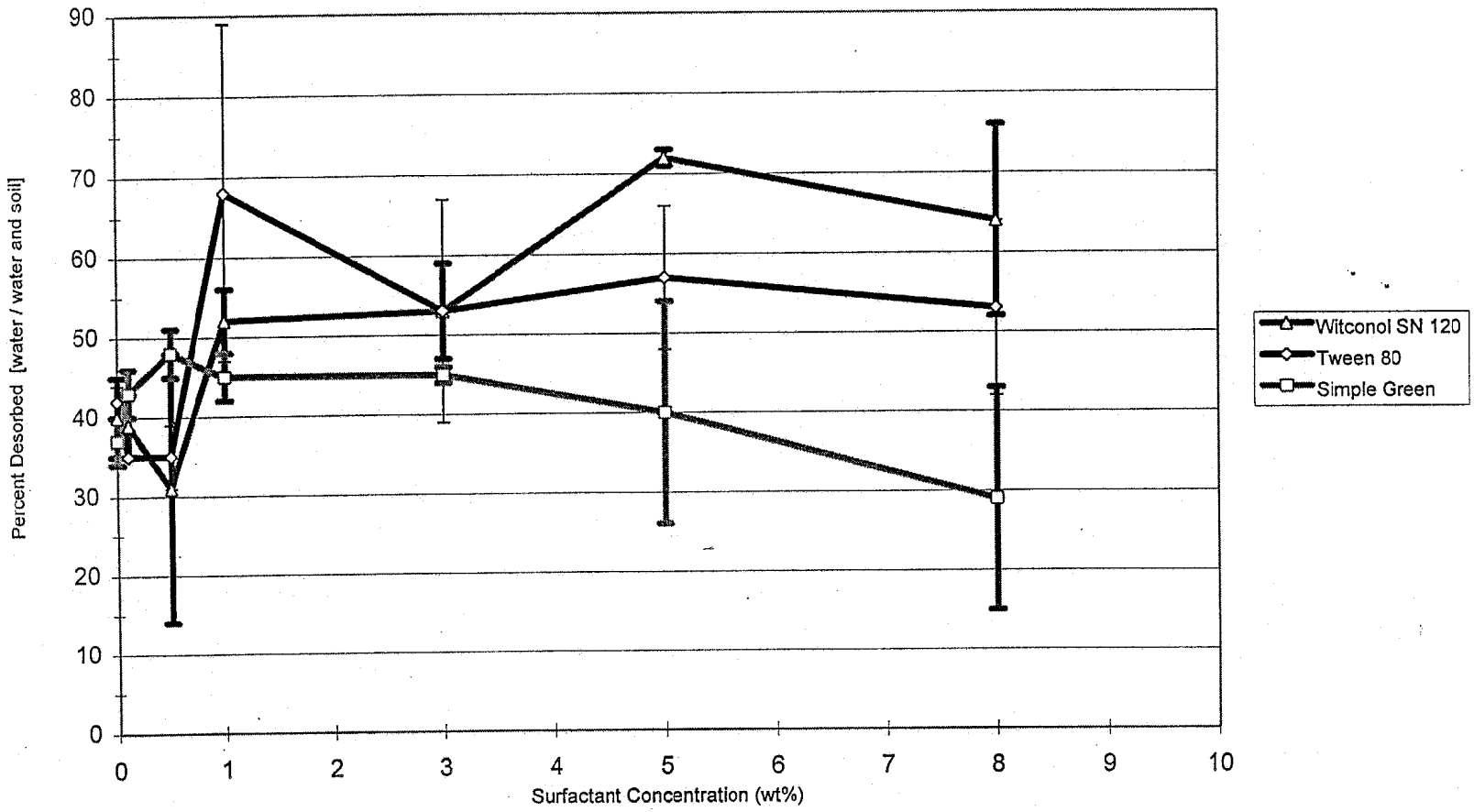

Figure 3-13. Comparison of three nonionic surfactants in the batch Desorption of TNT from soil.

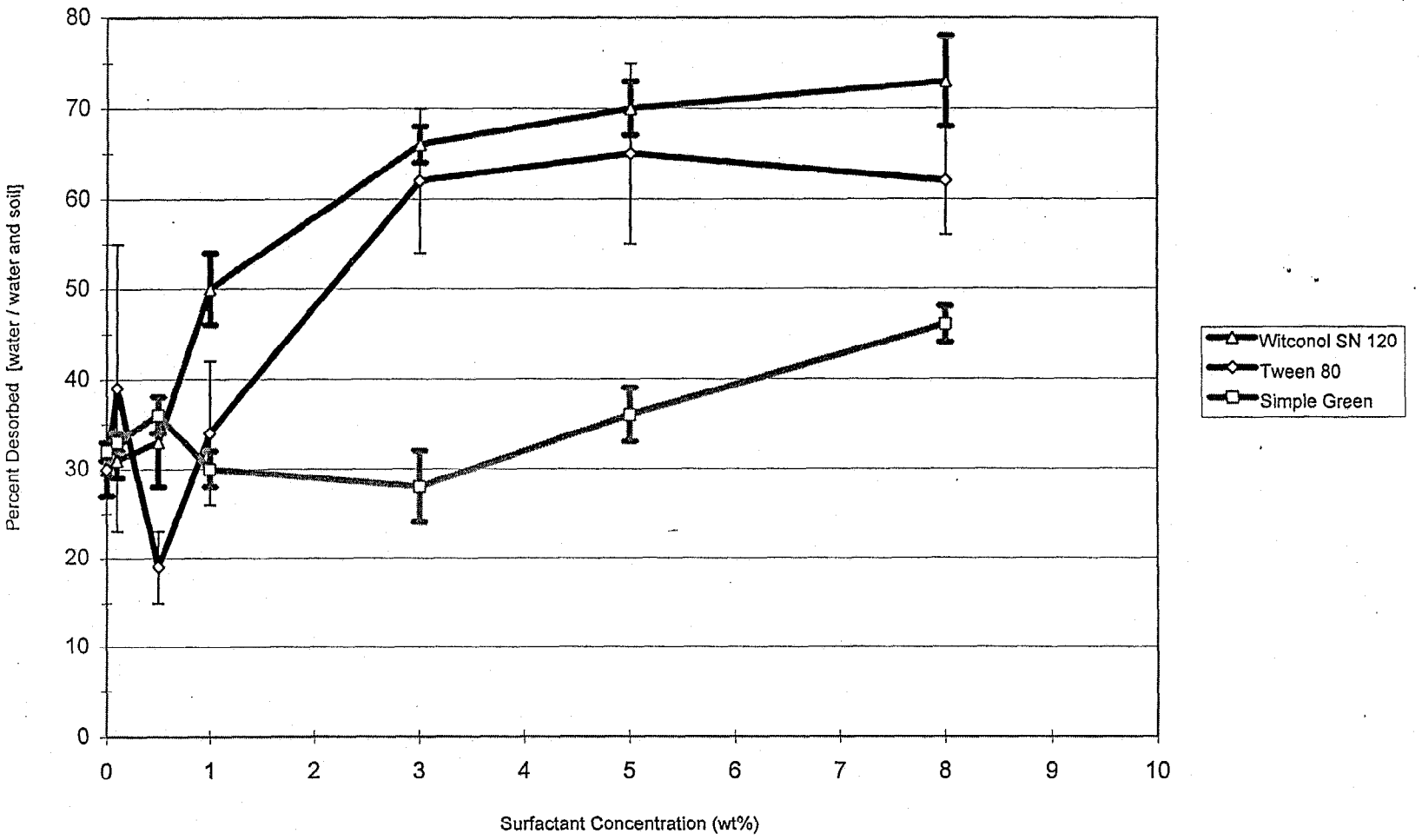

Figure 3-14. Comparison of three nonionic surfactants in the batch desorption of both 4A-DNT and 2A-DNT from soil.

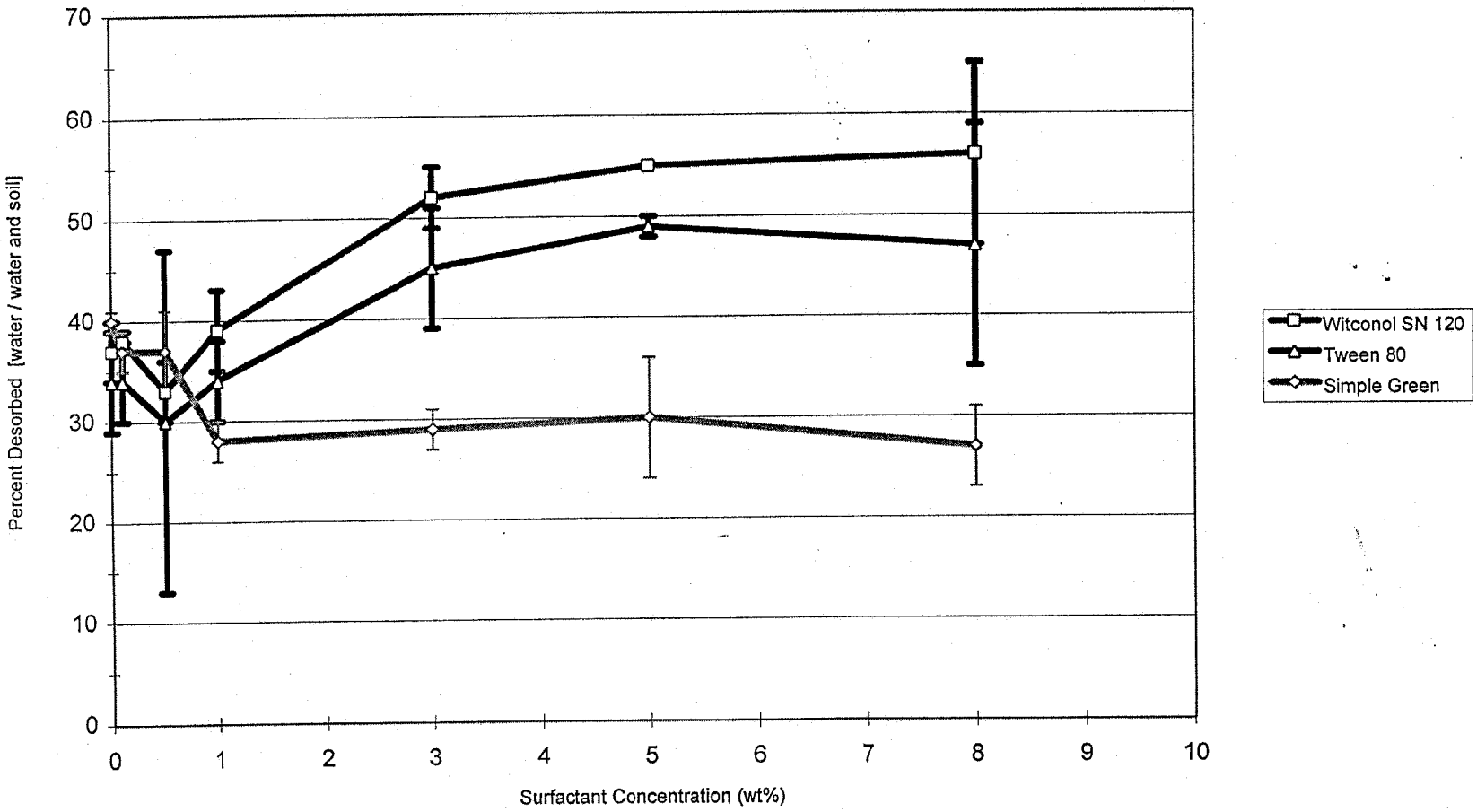

Figure 3-15. Comparison of three nonionic surfactants in the batch desorption of RDX from soil.

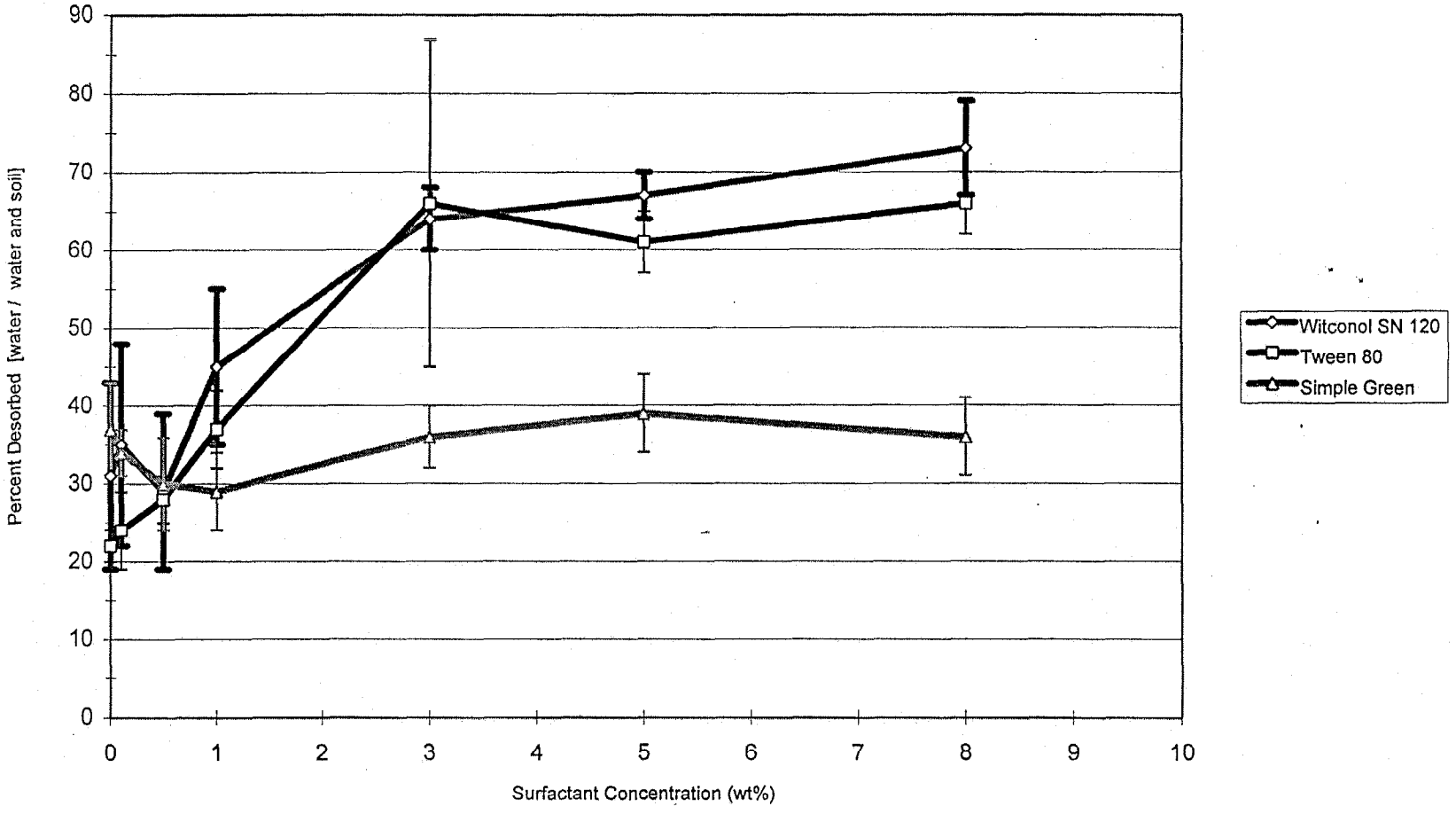

Figure 3-16. Comparison of three nonionic surfactants in the batch desorption of HMX from soil.

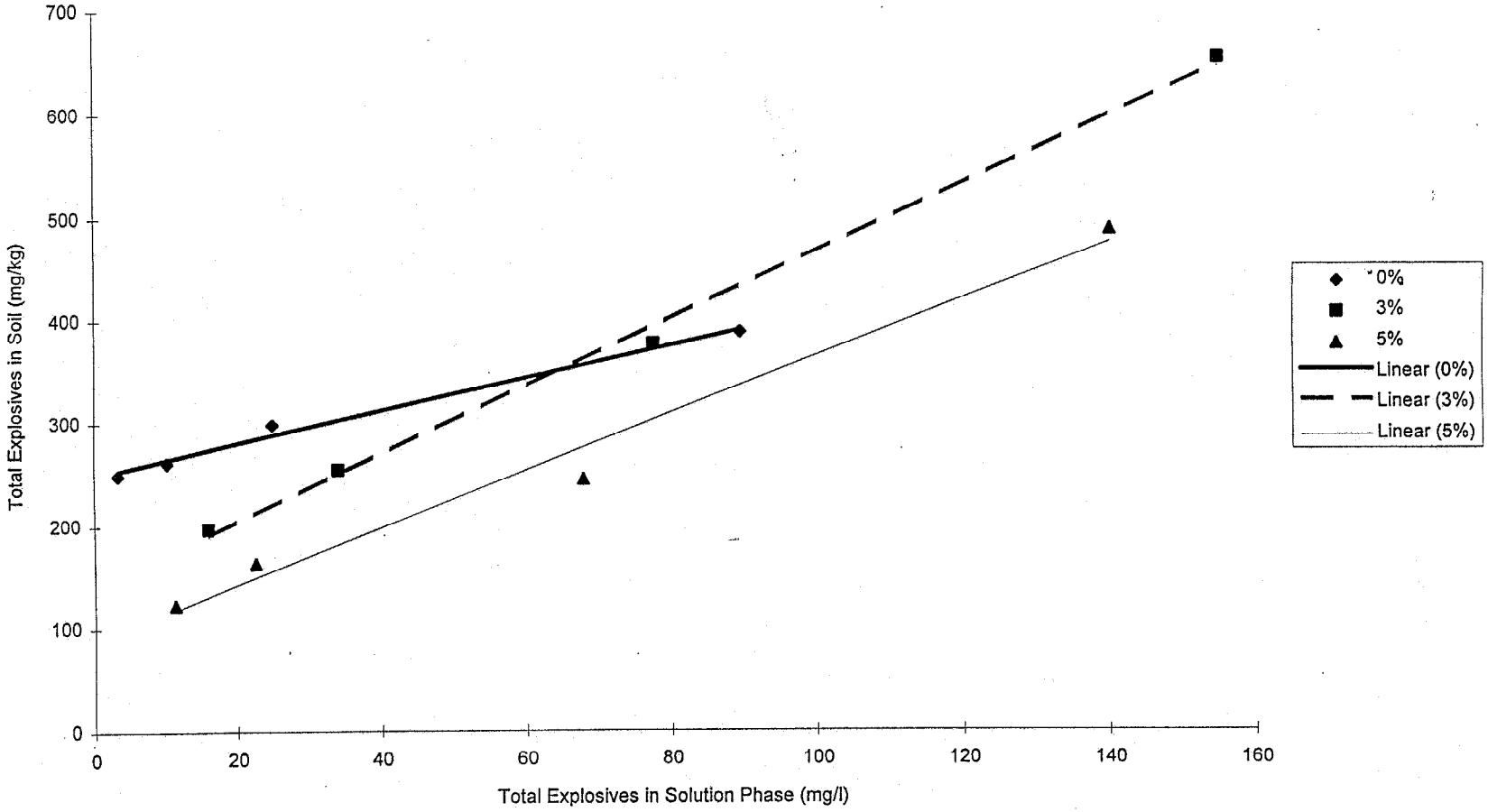

Figure 3-17. Linear isotherms of the sequential desorption of total explosives - comparison of Tween 80 and DDI water.

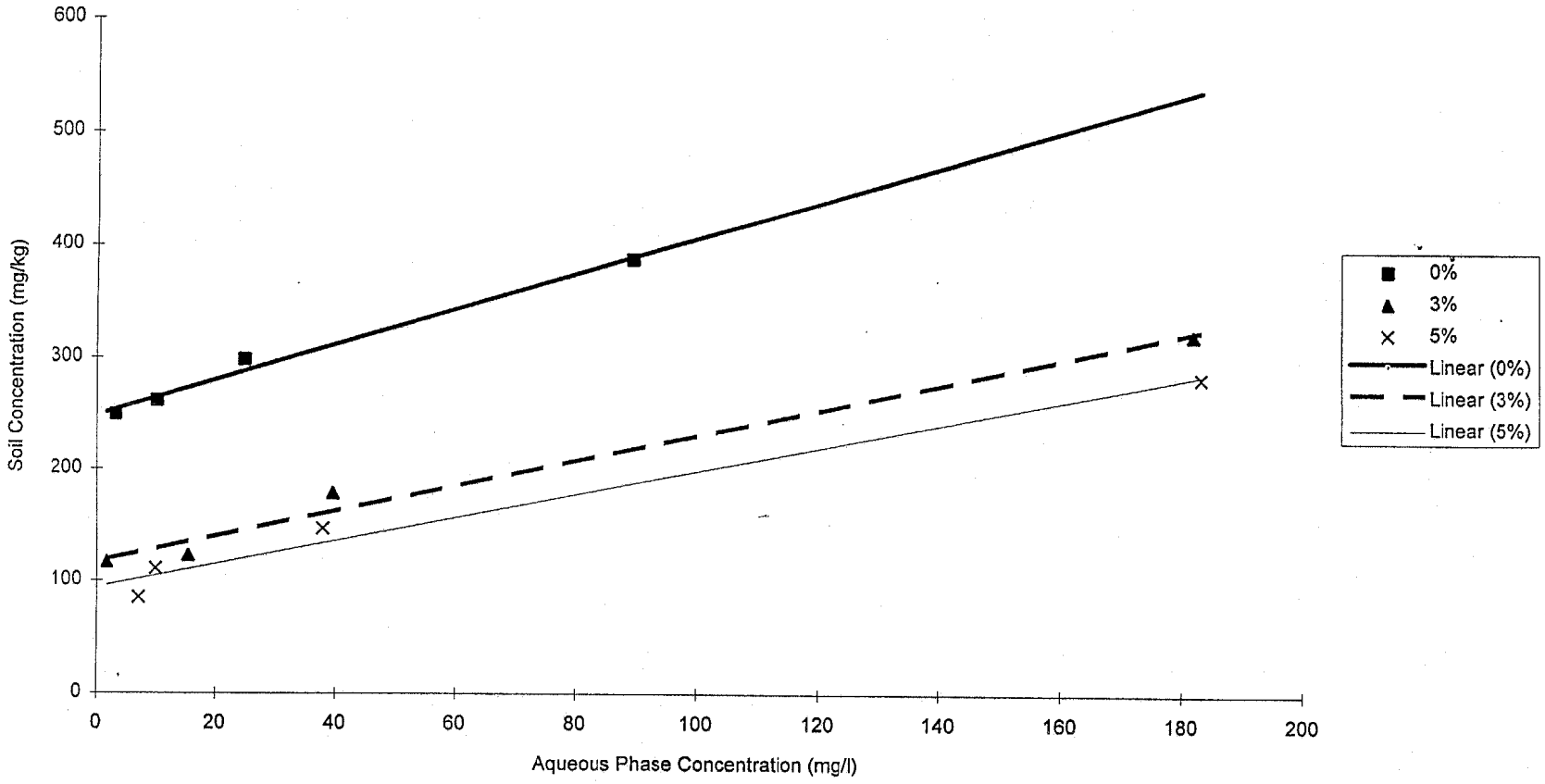

Figure 3-18. Linear isotherms of the sequential desorption of total explosives - comparison of Witconol SN 120 and DDI water.

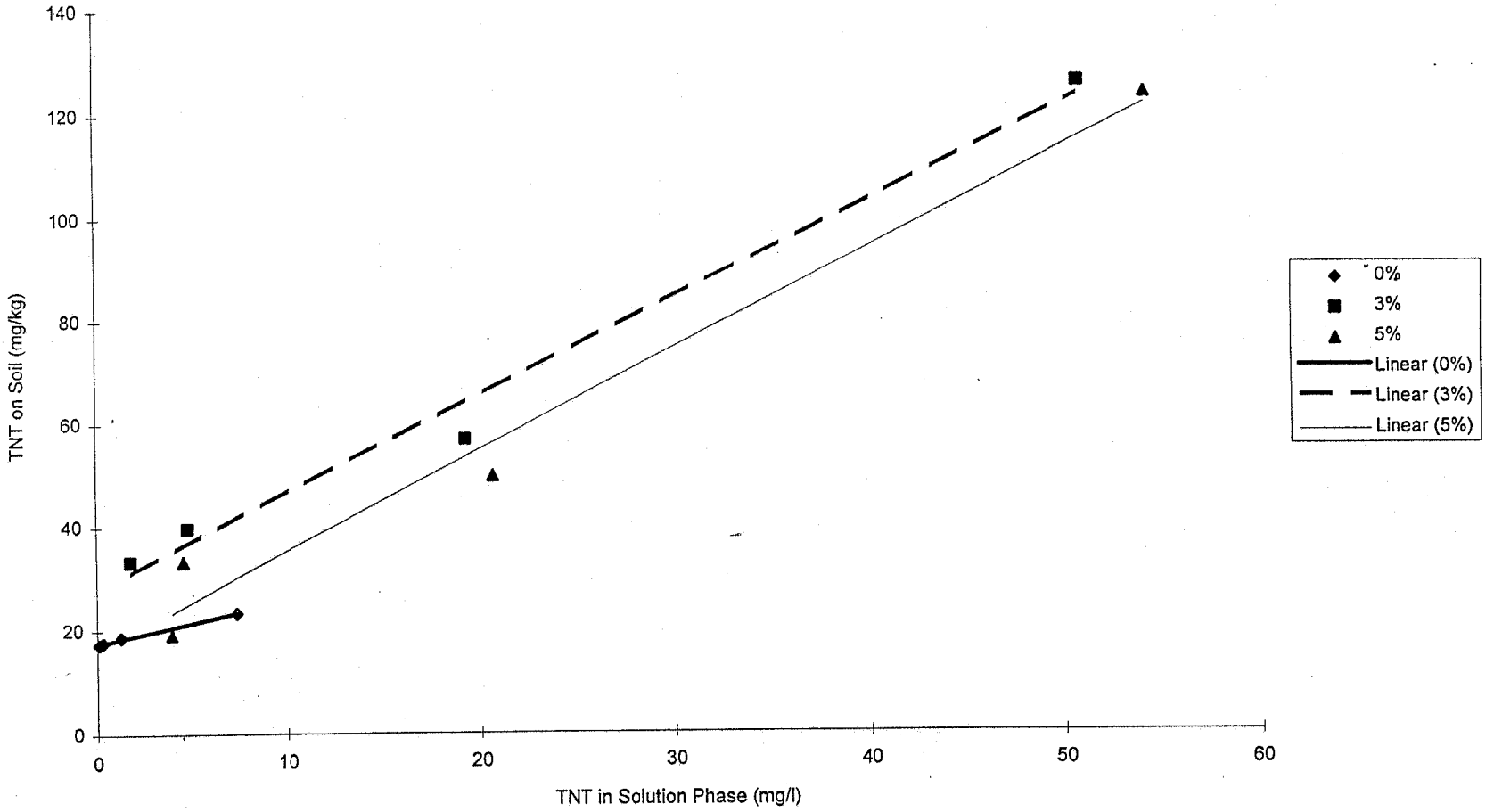

Figure 3-19. Linear isotherms of the sequential desorption of TNT from soil - comparison of Tween 80 and DDI water.

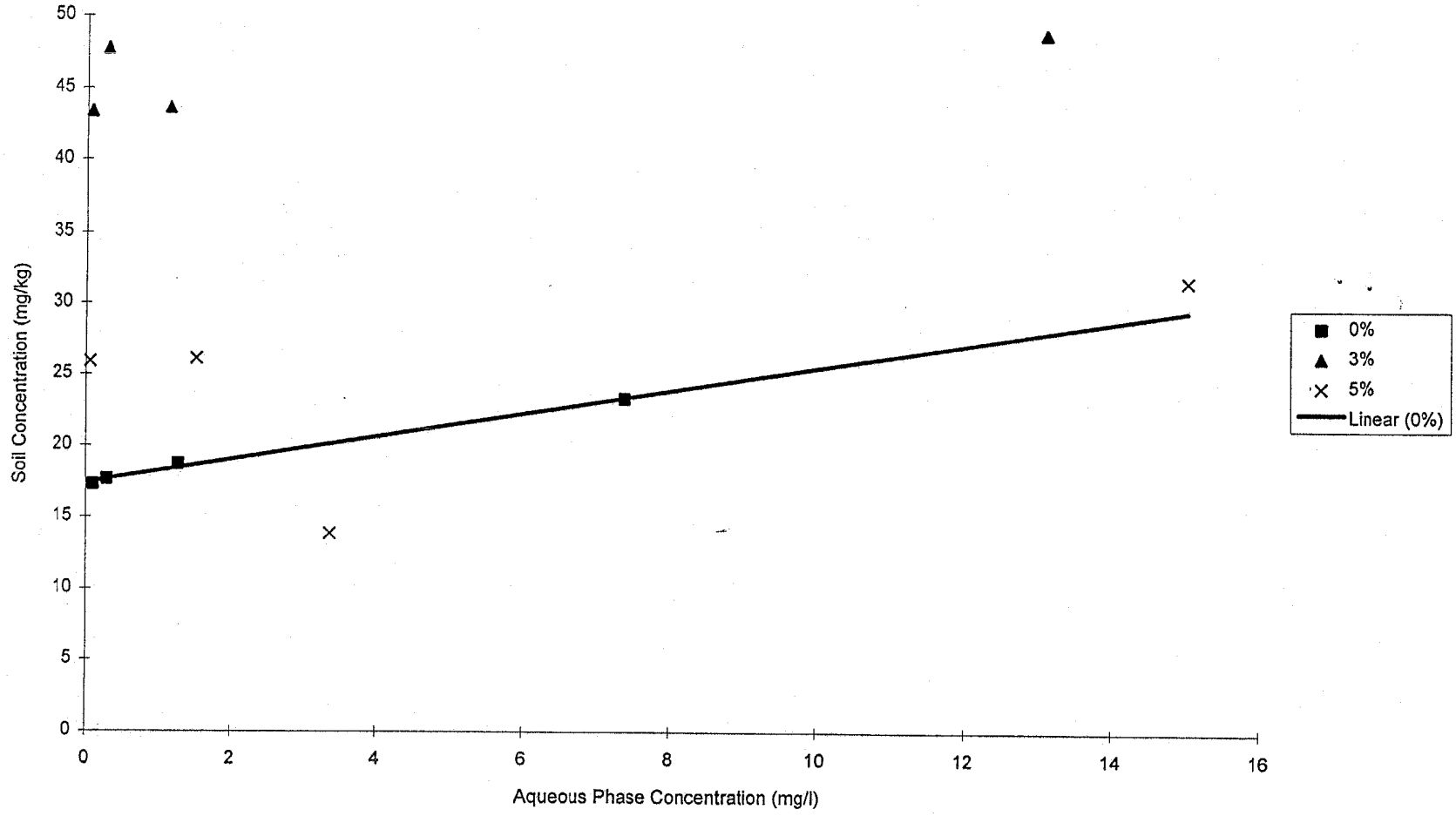

Figure 3-20. Linear isotherms of the sequential desorption of TNT from soil - comparsion of Witconol SN 120 and DDI water.

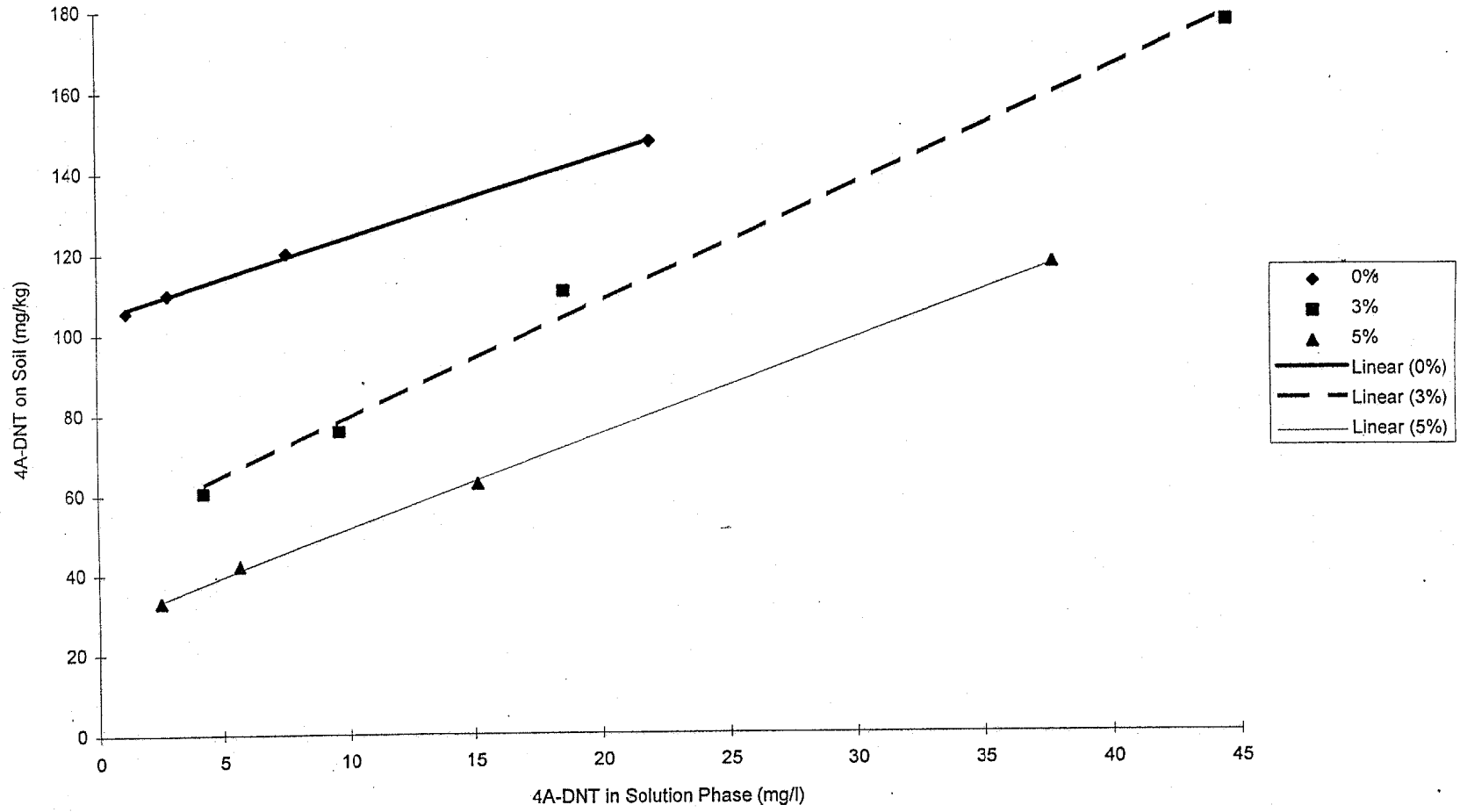

Figure 3-21. Linear desorption isotherms of the sequential desorption of 4A-DNT from soil - comparison of Tween 80 and DDI water.

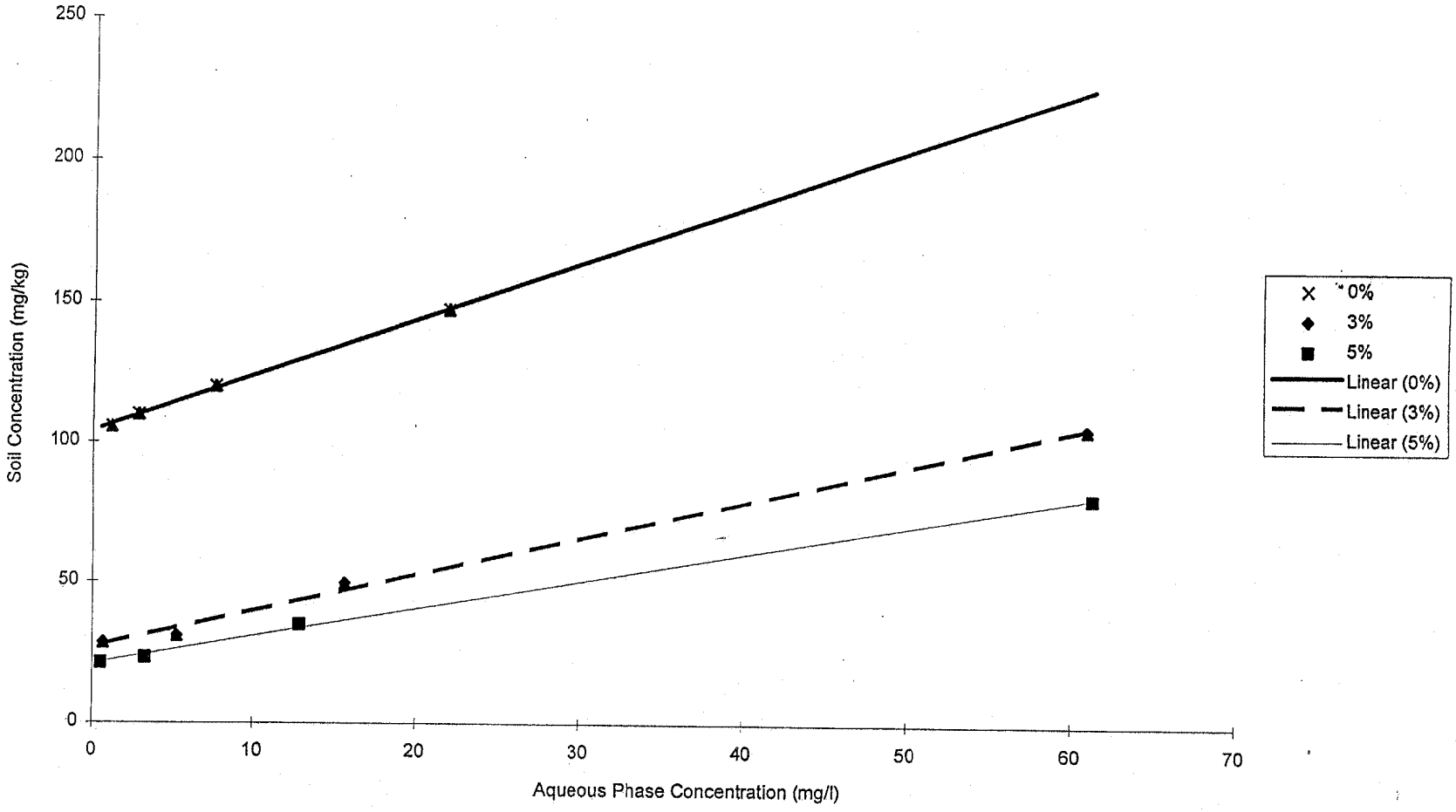

Figure 3-22. Linear isotherms of the sequential desorption of 4A-DNT from soil - comparison of Witconol SN 120 and DDI water.

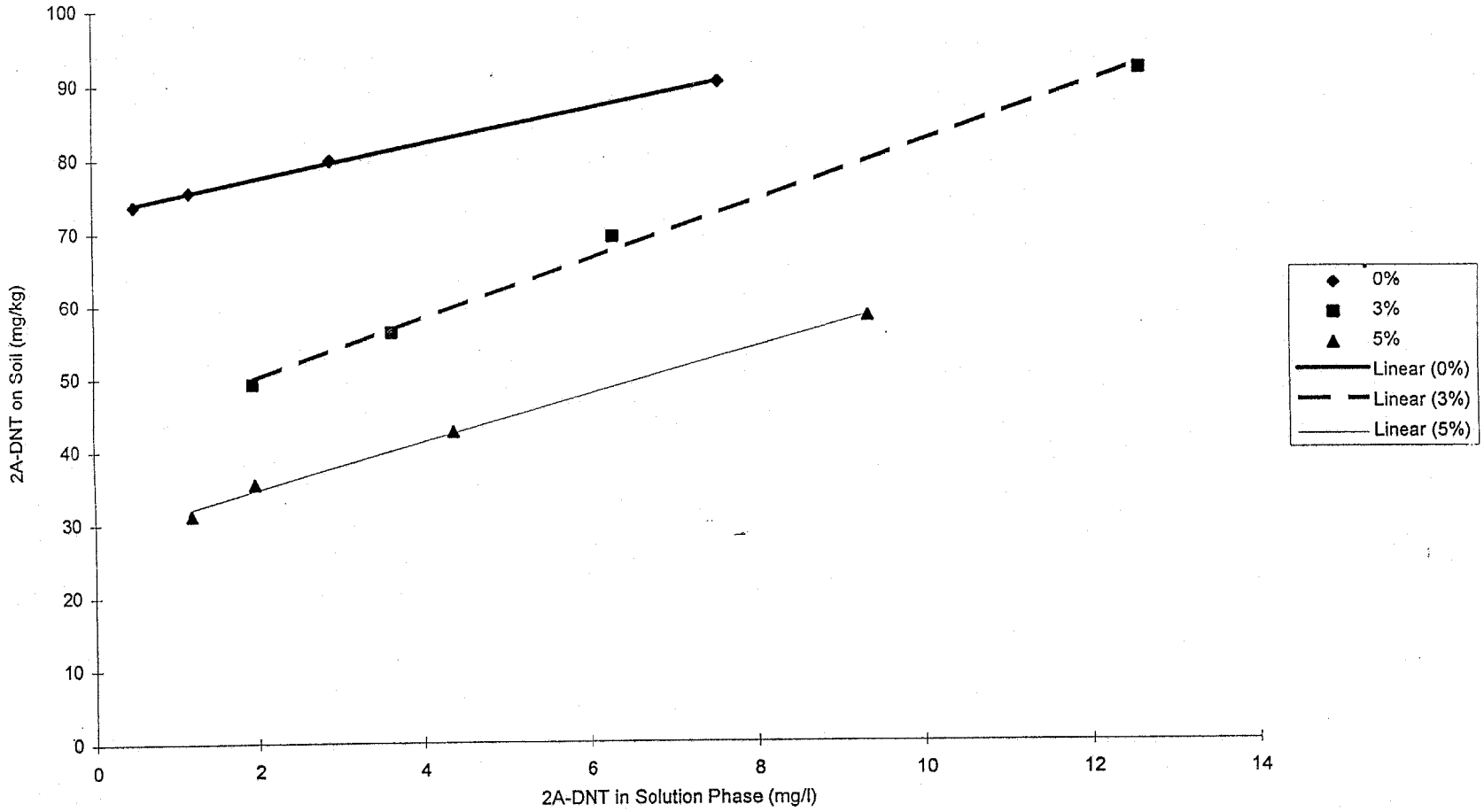

Figure 3-23. Linear isotherms of the sequential desorption of 2A-DNT from soil - comparison of Tween 80 and DDI water.

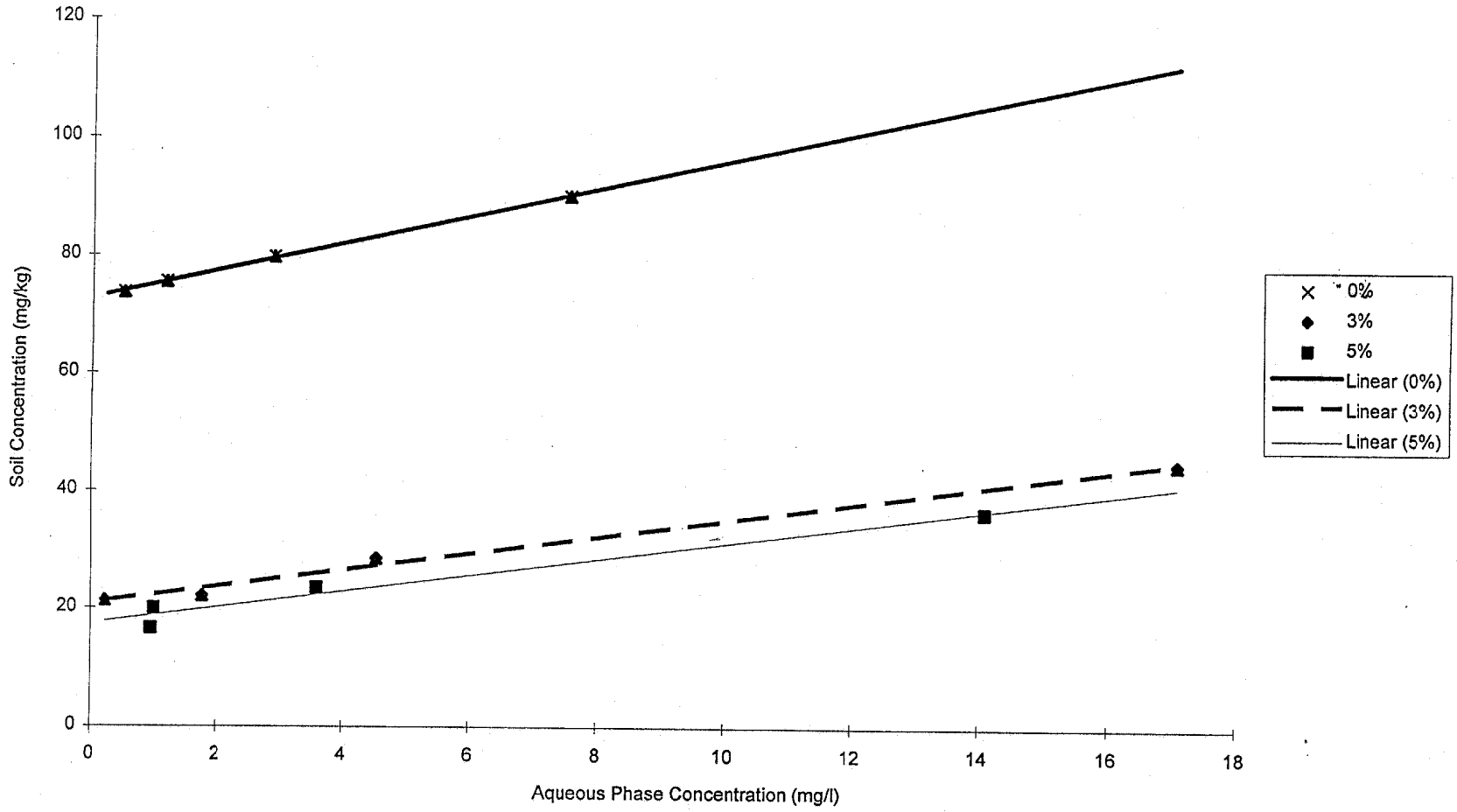

Figure 3-24. Linear isotherms of the sequential desorption of 2A-DNT from soil - comparison of Witconol SN 120 and DDI water.

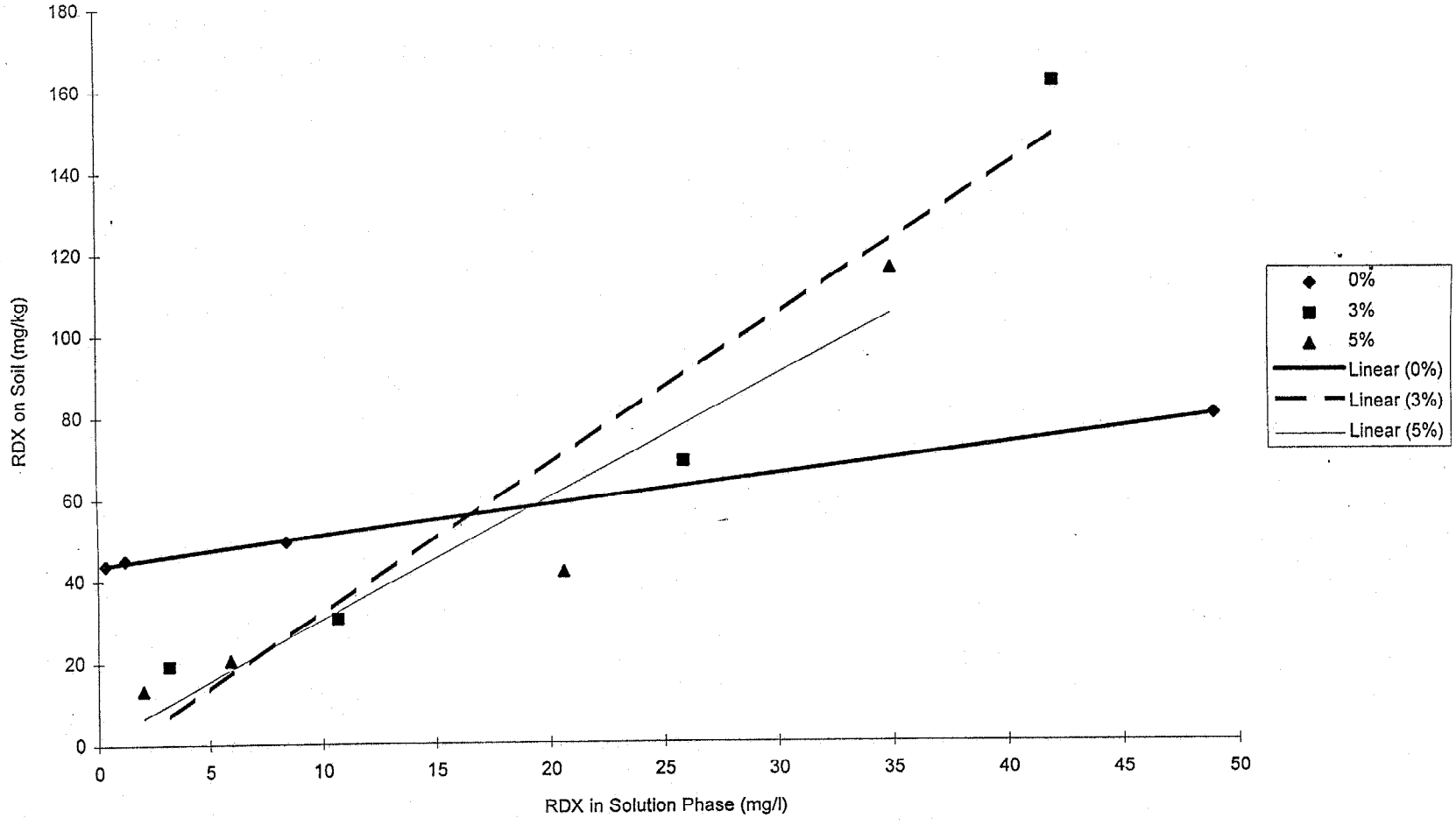

Figure 3-25. Linear isotherms of the sequential desorption of RDX from soil - comparison of Tween 80 and DDI water.

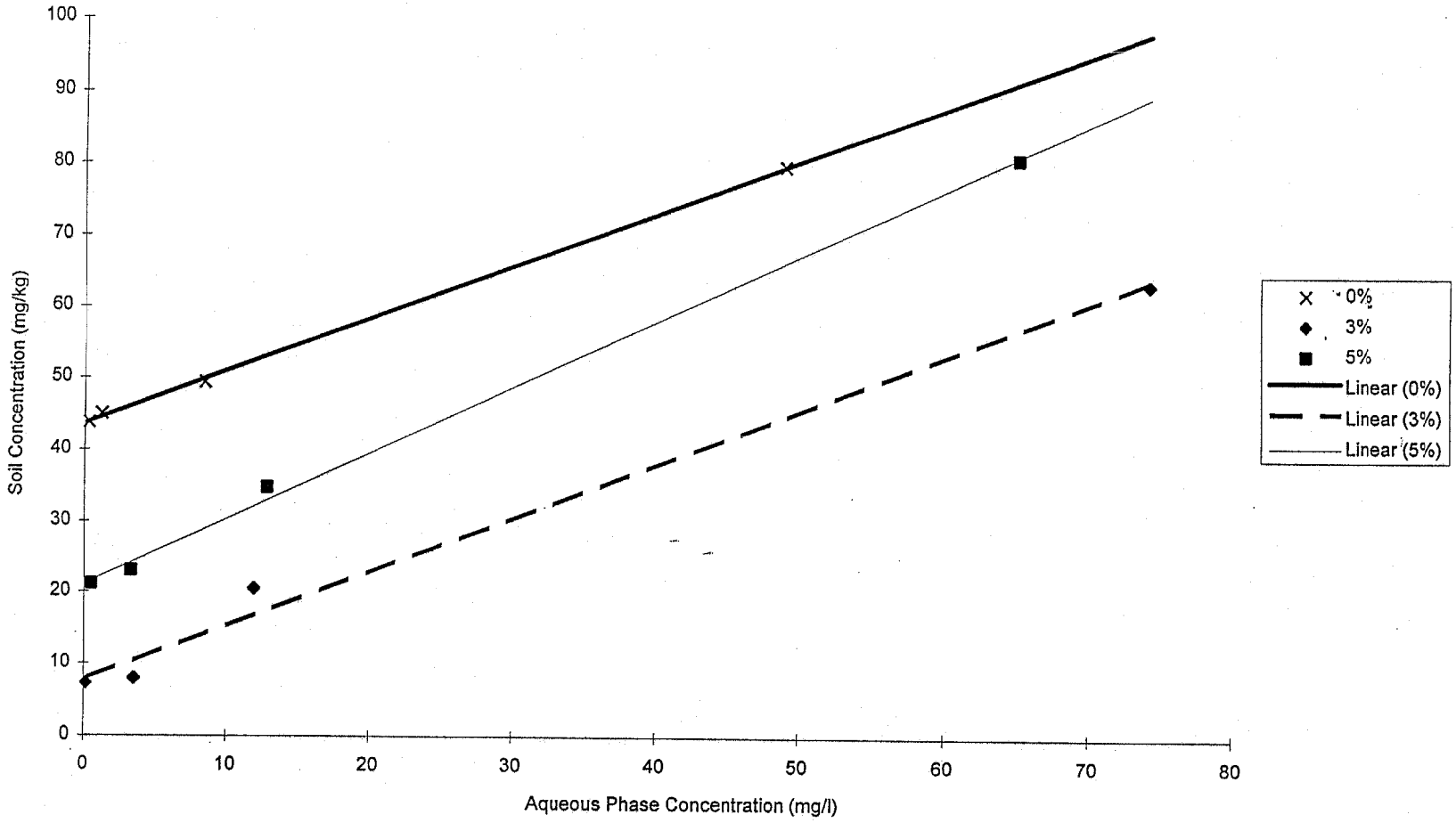

Figure 3-26. Linear isotherms of the sequential desorption of RDX from soil - comparison of Witconol SN 120 and DDI water.

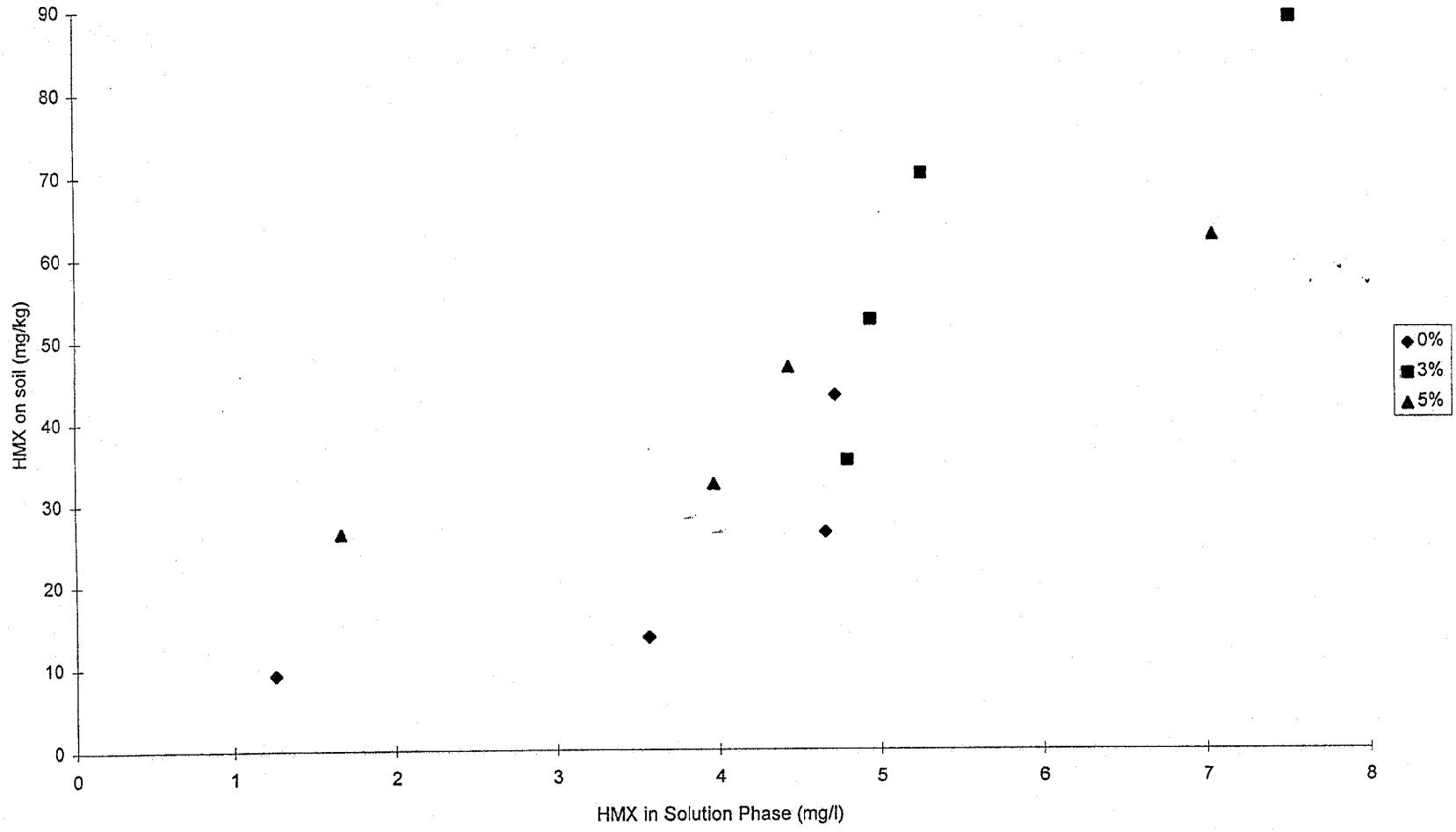

Figure 3-27. Linear isotherms of the sequential desorption of HMX from soil - comparison of Tween 80 and DDI water.

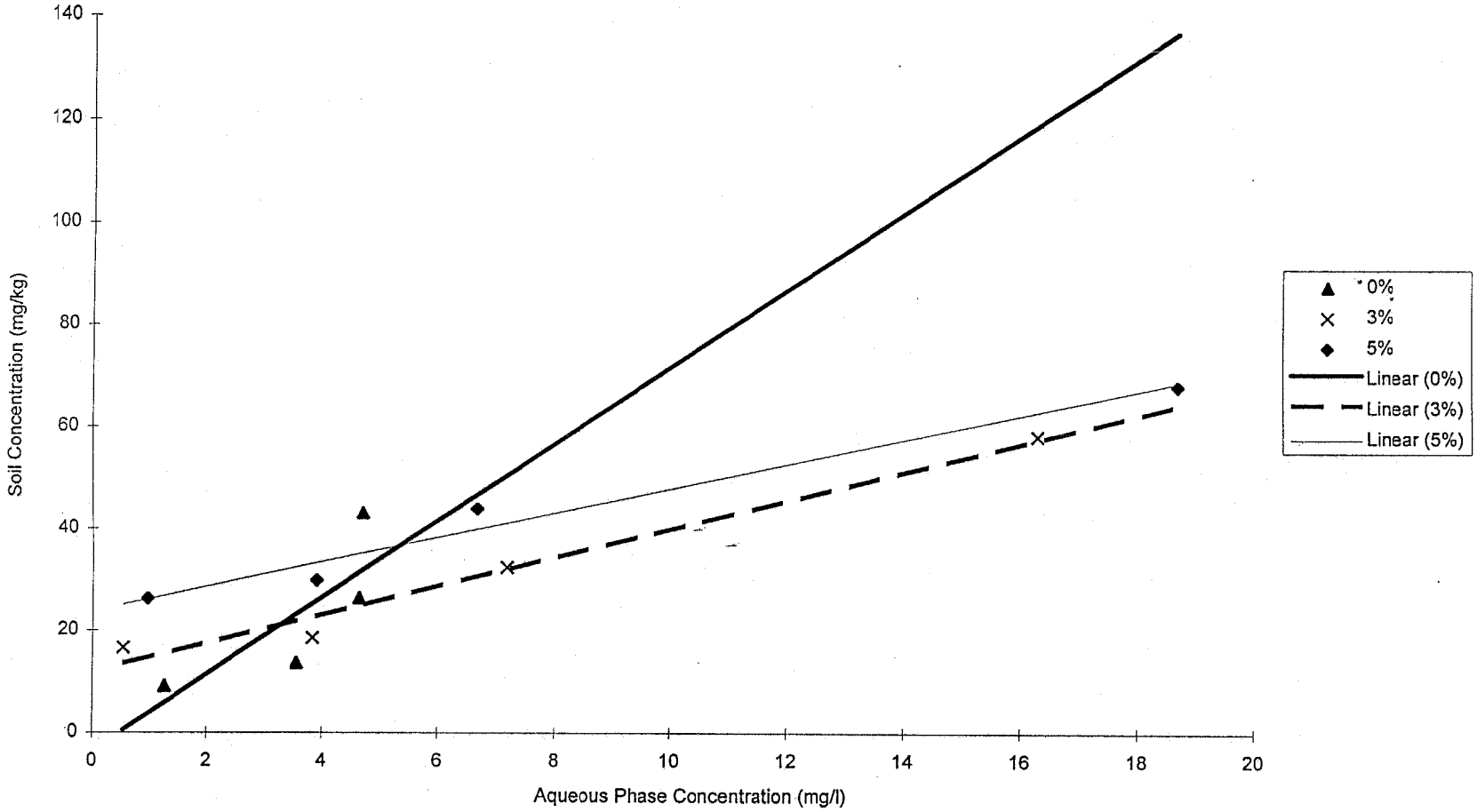

Figure 3-28. Linear isotherms of the sequential desorption of HMX from soil - comparison of Witconol SN 120 and DDI water.

Appendix A

Surface Tension: Raw Data

Table A-1. Time dependance of surface tension using Tween 80 in DDI water.

Concentration (mg/l)	Time (min)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)
0.00	2	76.0	22.0	1.16E-03	0.998	0.936	71.1
1.00	2	72.7	22.0	1.16E-03	0.998	0.933	67.8
5.00	2	69.2	22.0	1.16E-03	0.998	0.929	64.3
10.00	2	66.9	22.0	1.16E-03	0.998	0.927	62.0
15.00	2	65.6	22.0	1.16E-03	0.998	0.926	60.7
20.00	2	63.1	22.0	1.16E-03	0.998	0.923	58.3
50.00	2	44.7	22.0	1.16E-03	0.998	0.904	40.4
100.00	2	42.7	22.0	1.16E-03	0.998	0.901	38.5
1.00	5	70.2	22.0	1.16E-03	0.998	0.930	65.3
5.00	5	61.6	22.0	1.16E-03	0.998	0.922	56.8
10.00	5	59.0	22.0	1.16E-03	0.998	0.919	54.2
15.00	5	53.9	22.0	1.16E-03	0.998	0.914	49.2
20.00	5	52.4	22.0	1.16E-03	0.998	0.912	47.8
50.00	5	44.1	22.0	1.16E-03	0.998	0.903	39.8
100.00	5	40.5	22.0	1.16E-03	0.998	0.899	36.4
1.00	10	69.4	22.0	1.16E-03	0.998	0.930	64.5
5.00	10	60.3	22.0	1.16E-03	0.998	0.920	55.5
10.00	10	57.2	22.0	1.16E-03	0.998	0.917	52.5
15.00	10	53.0	22.0	1.16E-03	0.998	0.913	48.4
20.00	10	51.0	22.0	1.16E-03	0.998	0.911	46.4
50.00	10	43.2	22.0	1.16E-03	0.998	0.902	39.0
100.00	10	40.6	22.0	1.16E-03	0.998	0.899	36.5
1.00	15	68.2	22.0	1.16E-03	0.998	0.928	63.3
5.00	15	58.4	22.0	1.16E-03	0.998	0.918	53.6
10.00	15	53.6	22.0	1.16E-03	0.998	0.913	49.0
15.00	15	52.7	22.0	1.16E-03	0.998	0.912	48.1
20.00	15	50.5	22.0	1.16E-03	0.998	0.910	46.0

Table A-1 (Continued). Time dependance of surface tension using Tween 80 in DDI water.

Concentration (mg/l)	Time (min)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)
50.00	15	43.1	22.0	1.16E-03	0.998	0.902	38.9
100.00	15	40.9	22.0	1.16E-03	0.998	0.899	36.8
1.00	20	68.5	22.0	1.16E-03	0.998	0.929	63.6
5.00	20	58.8	22.0	1.16E-03	0.998	0.919	54.0
10.00	20	55.1	22.0	1.16E-03	0.998	0.915	50.4
15.00	20	54.0	22.0	1.16E-03	0.998	0.914	49.3
20.00	20	51.3	22.0	1.16E-03	0.998	0.911	46.7
50.00	20	43.3	22.0	1.16E-03	0.998	0.902	39.1
100.00	20	40.6	22.0	1.16E-03	0.998	0.899	36.5
1.00	30	67.6	22.0	1.16E-03	0.998	0.928	62.7
5.00	30	58.5	22.0	1.16E-03	0.998	0.919	53.7
10.00	30	54.9	22.0	1.16E-03	0.998	0.915	50.2
15.00	30	53.8	22.0	1.16E-03	0.998	0.914	49.2
20.00	30	51.2	22.0	1.16E-03	0.998	0.911	46.6
50.00	30	43.6	22.0	1.16E-03	0.998	0.902	39.3
100.00	30	40.8	22.0	1.16E-03	0.998	0.899	36.7
1.00	40	68.5	22.0	1.16E-03	0.998	0.929	63.6
5.00	40	58.4	22.0	1.16E-03	0.998	0.918	53.6
10.00	40	54.2	22.0	1.16E-03	0.998	0.914	49.5
15.00	40	53.7	22.0	1.16E-03	0.998	0.913	49.1
20.00	40	51.4	22.0	1.16E-03	0.998	0.911	46.8
50.00	40	43.6	22.0	1.16E-03	0.998	0.902	39.3
100.00	40	40.6	22.0	1.16E-03	0.998	0.899	36.5

Table A-2. Surface tension data: Tween 80 in DDI water.

Concentration (mg/l)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)	Average (dyn/cm)	Standard Deviation
0.00	76.3	22.0	1.16E-03	0.998	0.936	71.4		
0.00	76.0	22.0	1.16E-03	0.998	0.936	71.1		
0.00	76.2	22.0	1.16E-03	0.998	0.936	71.3	71.3	0.154
0.10	76.2	22.0	1.16E-03	0.998	0.936	71.3		
0.10	76.2	22.0	1.16E-03	0.998	0.936	71.3		
0.10	76.3	22.0	1.16E-03	0.998	0.936	71.4	71.4	0.0583
0.50	76.1	22.0	1.16E-03	0.998	0.936	71.2		
0.50	76.1	22.0	1.16E-03	0.998	0.936	71.2		
0.50	76.2	22.0	1.16E-03	0.998	0.936	71.3	71.3	0.0583
1.00	68.5	22.0	1.16E-03	0.998	0.928	63.6		
1.00	67.9	22.0	1.16E-03	0.998	0.928	63.0		
1.00	69.0	22.0	1.16E-03	0.998	0.929	64.1	63.6	0.549
5.00	58.6	22.0	1.16E-03	0.998	0.918	53.8		
5.00	58.1	22.0	1.16E-03	0.998	0.918	53.3		
5.00	59.6	22.0	1.16E-03	0.998	0.919	54.8	54.0	0.749
10.0	55.8	22.0	1.16E-03	0.998	0.915	51.1		
10.0	57.0	22.0	1.16E-03	0.998	0.917	52.3		
10.0	53.5	22.0	1.16E-03	0.998	0.913	48.8	50.7	1.73
15.0	54.0	22.0	1.16E-03	0.998	0.914	49.3		
15.0	54.0	22.0	1.16E-03	0.998	0.914	49.3		
15.0	53.5	22.0	1.16E-03	0.998	0.913	48.8	49.2	0.280
20.0	51.6	22.0	1.16E-03	0.998	0.911	47.0		
20.0	51.9	22.0	1.16E-03	0.998	0.911	47.3		
20.0	49.2	22.0	1.16E-03	0.998	0.908	44.7	46.3	1.43

Table A-2 (Continued). Surface tension data: Tween 80 in DDI water.

Concentration (mg/l)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)	Average (dyn/cm)	Standard Deviation
25.0	49.0	22.0	1.16E-03	0.998	0.908	44.5		
25.0	47.0	22.0	1.16E-03	0.998	0.906	42.6		
25.0	50.1	22.0	1.16E-03	0.998	0.909	45.6	44.2	1.51
50.0	47.8	22.0	1.16E-03	0.998	0.907	43.3		
50.0	47.3	22.0	1.16E-03	0.998	0.906	42.9		
50.0	49.4	22.0	1.16E-03	0.998	0.909	44.9	43.7	1.05
100	44.7	22.0	1.16E-03	0.998	0.903	40.4		
100	44.6	22.0	1.16E-03	0.998	0.903	40.3		
100	45.7	22.0	1.16E-03	0.998	0.904	41.3	40.7	0.581
1000	43.4	22.0	1.16E-03	0.998	0.902	39.1		
1000	42.8	22.0	1.16E-03	0.998	0.901	38.6		
1000	42.6	22.0	1.16E-03	0.998	0.901	38.4	38.7	0.396
10000	42.8	22.0	1.16E-03	0.998	0.901	38.6		
10000	42.0	22.0	1.16E-03	0.998	0.900	37.8		
10000	43.0	22.0	1.16E-03	0.998	0.901	38.8	38.4	0.502
100000	41.4	22.0	1.16E-03	0.998	0.899	37.2		
100000	41.3	22.0	1.16E-03	0.998	0.899	37.1		
100000	41.8	22.0	1.16E-03	0.998	0.900	37.6	37.3	0.251

Table A-3. Surface tension of aqueous extracts of Yorktown soil slurries dosed with Tween 80.

Dose (mg/l)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)	Average (dyn/cm)	Standard Deviation
0.00	63.8	22.0	1.16E-03	0.998	0.924	58.9		
0.00	65.4	22.0	1.16E-03	0.998	0.925	60.5		
0.00	63.3	22.0	1.16E-03	0.998	0.923	58.4	59.3	1.086
4.00	64.5	22.0	1.16E-03	0.998	0.924	59.6		
4.00	65.0	22.0	1.16E-03	0.998	0.925	60.1		
4.00	65.8	22.0	1.16E-03	0.998	0.926	60.9	60.2	0.6499
12.00	65.9	22.0	1.16E-03	0.998	0.926	61.0		
12.00	63.3	22.0	1.16E-03	0.998	0.923	58.4		
12.00	65.0	22.0	1.16E-03	0.998	0.925	60.1	59.9	1.3071
20.00	65.8	22.0	1.16E-03	0.998	0.926	60.9		
20.00	65.9	22.0	1.16E-03	0.998	0.926	61.0		
20.00	64.8	22.0	1.16E-03	0.998	0.925	59.9	60.6	0.603
30.00	64.2	22.0	1.16E-03	0.998	0.924	59.3		
30.00	65.4	22.0	1.16E-03	0.998	0.925	60.5		
30.00	63.8	22.0	1.16E-03	0.998	0.924	58.9	59.6	0.824
50.0	65.5	22.0	1.16E-03	0.998	0.925	60.6		
50.0	64.7	22.0	1.16E-03	0.998	0.925	59.8		
50.0	64.2	22.0	1.16E-03	0.998	0.924	59.3	59.9	0.65
100.0	65.5	22.0	1.16E-03	0.998	0.925	60.6		
100.0	65.5	22.0	1.16E-03	0.998	0.925	60.6		
100.0	60.1	22.0	1.16E-03	0.998	0.920	55.3	58.8	3.077
1000.0	58.7	22.0	1.16E-03	0.998	0.918	53.9		
1000.0	61.4	22.0	1.16E-03	0.998	0.921	56.6		
1000.0	60.6	22.0	1.16E-03	0.998	0.920	55.8	55.4	1.36

Table A-3 (Continued). Surface tension of aqueous extracts of Yorktown soil slurries dosed With Tween 80.

Dose (mg/l)	Dial reading (dyn/cm)	Temperature (Celsius)	Air Density (g/cm^3)	Water Density (g/cm^3)	Correction Factor	Surface Tension (dyn/cm)	Average (dyn/cm)	Standard Deviation
5000.0	59.6	22.0	1.16E-03	0.998	0.919	54.8		
5000.0	51.3	22.0	1.16E-03	0.998	0.911	46.7		
5000.0	57.4	22.0	1.16E-03	0.998	0.917	52.6	51.4	4.19
7000.0	61.0	22.0	1.16E-03	0.998	0.921	56.2		
7000.0	58.9	22.0	1.16E-03	0.998	0.919	54.1		
7000.0	49.8	22.0	1.16E-03	0.998	0.909	45.3	51.9	5.79
10000	44.9	22.0	1.16E-03	0.998	0.903	40.6		
10000	58.4	22.0	1.16E-03	0.998	0.918	53.6		
10000	55.3	22.0	1.16E-03	0.998	0.915	50.6	48.3	6.833
15000	47.0	22.0	1.16E-03	0.998	0.906	42.6		
15000	42.8	22.0	1.16E-03	0.998	0.901	38.6		
15000	42.6	22.0	1.16E-03	0.998	0.901	38.4	39.8	2.371
20000	44.7	22.0	1.16E-03	0.998	0.903	40.4		
20000	41.8	22.0	1.16E-03	0.998	0.900	37.6		
20000	40.4	22.0	1.16E-03	0.998	0.898	36.3	38.1	2.083
30000	31.5	22.0	1.16E-03	0.998	0.888	28.0		
30000	41.6	22.0	1.16E-03	0.998	0.900	37.4		
30000	37.4	22.0	1.16E-03	0.998	0.895	33.5	32.9	4.755
50000	31.0	22.0	1.16E-03	0.998	0.887	27.5		
50000	41.0	22.0	1.16E-03	0.998	0.899	36.9		
50000	36.5	22.0	1.16E-03	0.998	0.894	32.6	32.3	4.689
100000	31.4	22.0	1.16E-03	0.998	0.887	27.9		
100000	38.7	22.0	1.16E-03	0.998	0.896	34.7		
100000	36.2	22.0	1.16E-03	0.998	0.893	32.3	31.6	3.465

Appendix B

Batch Desorption Data

Table B-1. Batch desorption explosives data: Tween 80

WATER Concentrations (mg/l)

SAMPLE	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	9.16	62.7	0.26	31.4	28.4	9.53	37.93	141.45
0%-B	9.09	65.3	0.23	38.2	30	10.2	40.2	153.02
0%-C	8.53	68.6	0.12	34.4	33.6	10	43.6	155.25
0.1%-A	7.88	63.4	0	11.6	21.7	7.8	29.5	112.38
0.1%-B	11.9	62.2	0.12	19.3	25.5	8.79	34.29	127.81
0.1%-C	12	63.4	0	18.8	27.4	91.2	118.6	212.8
0.5%-A	11.5	55.6	0	11.6	17.5	6.35	23.85	102.55
0.5%-B	11.3	60.2	0.28	18.7	18.3	6.6	24.9	115.38
0.5%-C	12.4	63.9	0.23	12.5	19.1	6.85	25.95	114.98
1.0%-A	12.1	47.4	0	52.9	27.9	9.45	37.35	149.75
1.0%-B	12.8	55	0.1	81.4	24.8	9.31	34.11	183.41
1.0%-C	12	57.9	0.21	22.8	29.1	10.7	39.8	132.71
3.0%-A	20.4	70.9	0.1	11	54.5	18.3	72.8	175.2
3.0%-B	16.7	76.2	0.17	28.8	58.6	21.6	80.2	202.07
3.0%-C	21.7	72.3	0.13	16.8	59.4	20.6	80	190.93
5.0%-A	24.2	70.4	0.16	23.1	63	22.6	85.6	203.46
5.0%-B	24.1	81.3	0.16	27	65.4	22.3	87.7	220.26
5.0%-C	22.6	76	0.15	23.1	64.6	22.1	86.7	208.55
8.0%-A	27.3	87	0.13	22.8	72.2	24.9	97.1	234.33
8.0%-B	26.7	77.3	0.14	38.6	64.8	25.1	89.9	232.64
8.0%-C	28.2	88	0.1	32.5	75.2	25.5	100.7	249.5
10.0%-A	29.5	87.7	0.7	17.5	69.6	22	91.6	227
10.0%-B	29.8	91.2	0	18.9	69.9	22	91.9	231.8
10.0%-C	28.1	86.2	0	17	70	22	92	223.3
15.0%-A	15.7	63.8	0	18.7	43	13	56	154.2
15.0%-B	32.2	85.9	0	26.7	69.2	23.9	93.1	237.9
15.0%-C	32.5	87	0	24.4	75.5	24.5	100	243.9
20.0%-A	29.8	79.3	0	39.7	61.9	20.7	82.6	231.4
20.0%-B	33.1	87.3	0.8	55	64.9	24	88.9	265.1
20.0%-C	30.6	80.1	0	16.3	75.8	22.5	98.3	225.3

Table B-1 (Continued). Batch desorption explosives data: Tween 80

Soil Concentrations (mg/kg)

SAMPLE	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	124	147	1.92	162	194	84	278	744.32
0%-B	98	140	4.24	167	240	138	378	825.44
0%-C	93.5	99	1.18	156	212	88.5	300.5	684.58
0.1%-A	100	104	1.074	71.00	139	61.5	200.5	476.574
0.1%-B	92.5	122	1.31	115.00	208	91.5	299.5	630.31
0.1%-C	148	137	0.99	116.00	204	86.5	290.5	692.49
0.5%-A	94.5	136	1.69	72.50	262	108	370	674.69
0.5%-B	114	170	2.35	136.00	321	135	456	878.35
0.5%-C	90	127	1.18	66.50	209	74.7	283.7	568.38
1.0%-A	88.5	144	1.61	69.50	258	121	379	682.61
1.0%-B	62.5	79.5	0.645	41.70	116	52.2	168.2	352.545
1.0%-C	66	104	1.2	93.50	168	70.7	238.7	503.4
3.0%-A	59.5	110	0.91	60.80	144	70.2	214.2	445.41
3.0%-B	45.8	89	2.95	62.80	97.9	43.8	141.7	342.25
3.0%-C	7.5	70	0.645	35.00	91.5	39.4	130.9	244.045
5.0%-A	42	72	0.705	58.50	110	63	173	346.205
5.0%-B	52.5	61	1	45.40	60	27.6	87.6	247.5
5.0%-C	57	106	1.02	81.00	139	80	219	464.02
8.0%-A	55.5	107	3.78	105.00	164	94	258	529.28
8.0%-B	46.4	94.5	1.12	115.00	112	70	182	439.02
8.0%-C	40.4	79.5	0.82	59.50	103	62.5	165.5	345.72
10.0%-A	63.2	67.5	1.53	37.90	87	52.4	139.4	309.53
10.0%-B	43.2	58.1	1.17	28.10	75.9	40.8	116.7	247.27
10.0%-C	51.8	63.2	1.16	32.50	76	38	114	262.66
15.0%-A	67.6	110	1.18	64.70	148	62.1	210.1	453.58
15.0%-B	26.1	48.8	0.215	13.60	59.9	26.1	86	174.715
15.0%-C	44.6	55.8	1.9	16.60	85.9	43.8	129.7	248.6
20.0%-A	35.5	51.5	0.615	26.00	91.7	41.6	133.3	246.915
20.0%-B	23.8	42.7	1.02	36.20	46.7	22.3	69	172.72
20.0%-C	23.5	42.9	1.03	18.90	62.1	30.3	92.4	178.73

Table B-2. Batch desorption explosives data: Witconol SN 120.

Water Concentrations (mg/l)

Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	15.00	69.4	0.40	35.00	25.4	9.49	34.9	155
0%-B	7.38	65.0	0.18	14.70	27.4	8.98	36.4	124
0%-C	14.60	67.0	0.26	18.80	25.2	9.03	34.2	135
0.1%-A	14.40	50.7	0.28	23.20	24.5	9.19	33.7	122
0.1%-B	8.88	64.7	0.15	7.24	27.1	9.06	36.2	117
0.1%-C	14.30	59.1	0.21	11.10	30.3	10.40	40.7	125
0.5%-A	10.40	51.6	0.29	5.97	30.3	10.40	40.7	109
0.5%-B	15.50	50.6	0.23	0.99	27.4	10.00	37.4	105
0.5%-C	9.50	51.2	0.28	3.62	26.4	9.61	36.0	101
1.0%-A	17.20	54.5	0.25	6.89	42.1	15.40	57.5	136
1.0%-B	13.60	60.0	0.34	9.29	42.1	15.30	57.4	141
1.0%-C	16.00	53.6	0.25	15.10	39.7	14.80	54.5	139
3.0%-A	21.70	58.6	0.39	5.73	52.8	20.20	73.0	159
3.0%-B	22.40	59.5	0.19	23.40	53.4	20.20	73.6	179
3.0%-C	21.20	60.3	0.30	4.87	52.8	19.80	72.6	159
5.0%-A	25.90	69.6	0.30	12.10	65.2	25.00	90.2	198
5.0%-B	24.40	66.5	0.24	10.70	61.9	23.40	85.3	187
5.0%-C	25.50	68.7	0.36	22.70	63.9	23.70	87.6	205
8.0%-A	27.60	68.2	0.28	12.90	67.8	26.50	94.3	203
8.0%-B	25.80	60.7	0.24	8.92	60.1	23.90	84.0	180
8.0%-C	23.30	50.8	0.11	3.26	52.2	34.40	86.6	164
10.0%-A	13.50	60.5	0.00	13.00	27.1	8.90	36.0	123
10.0%-B	24.30	79.6	0.00	14.50	71.9	23.80	95.7	214
10.0%-C	8.90	57.6	0.00	2.60	29.5	9.40	38.9	108
15.0%-A	16.40	64.2	0.40	6.30	41.0	13.40	54.4	142
15.0%-B	24.10	78.8	0.50	21.60	69.1	23.20	92.3	217
15.0%-C	21.30	73.9	0.50	14.00	65.2	22.00	87.2	197
20.0%-A	22.50	77.8	0.50	11.60	69.8	22.70	92.5	205
20.0%-B	23.80	80.0	0.40	18.40	75.1	24.50	99.6	222
20.0%-C	22.00	76.1	0.40	20.70	68.6	22.40	91.0	210

Table B-2 (Continued). Batch desorption explosives data: Witconol SN 120.

Soil Concentrations (mg/kg)

Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	90.3	129.0	3.34	145.0	194.0	100.0	294	662
0%-B	116.0	115.0	2.02	89.4	209.0	101.0	310	632
0%-C	71.9	97.7	2.37	95.4	159.0	76.5	236	503
0.1%-A	59.2	87.3	1.93	104.0	153.0	80.7	234	486
0.1%-B	115.0	103.0	3.08	44.4	195.0	101.0	296	561
0.1%-C	73.4	90.9	2.82	58.1	205.0	108.0	313	538
0.5%-A	97.5	101.0	1.98	26.7	182.0	79.8	262	489
0.5%-B	77.7	119.0	7.02	24.3	222.0	107.0	329	557
0.5%-C	125.0	93.7	1.44	19.1	141.0	62.5	204	443
1.0%-A	52.6	70.0	1.74	22.8	105.0	54.2	159	306
1.0%-B	91.0	108.0	3.02	30.9	140.0	70.9	211	444
1.0%-C	57.0	91.5	1.98	38.3	133.0	61.9	195	384
3.0%-A	48.5	63.6	2.38	16.8	89.6	47.7	137	269
3.0%-B	39.8	50.0	1.92	91.9	73.3	40.0	113	297
3.0%-C	34.7	54.4	1.00	11.4	82.1	43.4	126	227
5.0%-A	35.1	56.8	1.70	16.0	79.0	44.9	124	234
5.0%-B	44.5	53.5	1.05	14.9	70.4	35.7	106	220
5.0%-C	42.6	56.0	1.84	27.8	91.6	54.0	146	274
8.0%-A	24.9	39.8	0.94	12.6	59.7	31.4	91.1	169
8.0%-B	41.0	73.4	6.08	20.1	84.4	48.2	133	273
8.0%-C	30.2	35.6	0.90	9.4	60.2	36.4	96.6	173
10.0%-A	52.6	62.3	1.26	32.7	126.0	52.9	179	328
10.0%-B	45.7	61.9	1.44	24.9	72.1	37.5	110	244
10.0%-C	70.8	90.3	1.85	29.0	178.0	92.1	270	462
15.0%-A	52.8	86.7	2.82	30.7	140.0	79.7	220	393
15.0%-B	41.5	52.0	2.25	25.3	63.3	32.7	96.0	217
15.0%-C	57.3	60.7	1.62	27.9	78.4	37.8	116	264
20.0%-A	48.6	67.2	1.42	31.1	90.1	45.5	136	284
20.0%-B	43.1	56.6	2.26	28.0	87.0	52.3	139	269
20.0%-C	49.9	61.4	1.80	31.8	85.4	45.1	131	275

Table B-3. Batch desoprtion explosives data: Simple Green.

Water Concentrations (mg/l)

SAMPLE	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	15.0	53.4	0.24	14.6	27.4	10.5	37.9	121
0%-B	14.0	68.7	0.27	31.9	30.0	10.6	40.6	155
0%-C	11.8	62.3	0.21	23.9	31.7	11.5	43.2	141
0.1%-A	12.8	61.0	0.20	29.6	30.6	11.7	42.3	146
0.1%-B	12.3	59.6	0.22	34.5	27.9	12.2	40.1	147
0.1%-C	11.5	61.8	0.30	47.4	34.5	12.1	46.6	168
0.5%-A	13.0	58.7	0.33	58.4	34.1	11.7	45.8	176
0.5%-B	10.2	62.6	0.18	28.5	30.7	11.6	42.3	144
0.5%-C	11.3	66.1	0.35	37.6	34.3	12.5	46.8	162
1.0%-A	10.0	59.0	0.19	30.3	34.6	11.4	46.0	145
1.0%-B	12.2	62.5	0.40	33.8	33.9	11.7	45.6	155
1.0%-C	9.9	51.8	0.28	44.3	26.6	10.2	36.8	143
3.0%-A	13.3	46.8	0.34	12.2	27.6	12.1	39.7	112
3.0%-B	13.8	55.0	0.40	15.3	29.9	12.1	42.0	127
3.0%-C	13.0	59.3	0.59	35.6	25.2	11.0	36.2	145
5.0%-A	13.8	51.6	0.44	3.2	35.2	17.1	52.3	121
5.0%-B	13.0	53.8	0.66	29.3	31.8	17.7	49.5	146
5.0%-C	15.9	61.5	1.11	21.8	33.2	17.4	50.6	151
8.0%-A	14.7	50.5	0.54	2.1	46.3	30.5	76.8	145
8.0%-B	15.2	56.2	1.04	10.6	43.0	31.4	74.4	157
8.0%-C	11.1	40.7	0.54	2.1	38.5	34.4	72.9	127

Table B-3 (Continued). Batch desoprtion explosives data: Simple Green.

Soil Concentrations (mg/kg)

SAMPLE	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	Total Aminos	Total Explosives
0%-A	62.0	81.6	2.35	94.8	185	97.1	282	523
0%-B	91.7	107	1.75	168	199	95.9	295	663
0%-C	77.0	88.0	1.75	127	196	102	298	592
0.1%-A	75.5	93.7	1.65	124	168	93.2	261	556
0.1%-B	81.2	104	1.95	172	176	103	279	638
0.1%-C	85.9	116	1.85	195	212	103	315	714
0.5%-A	78.5	124	1.80	186	186	91.2	277	668
0.5%-B	115	102	1.65	119	177	81.4	258	596
0.5%-C	83.8	99	1.65	137	170	79.1	249	570
1.0%-A	95.7	151	1.05	111	226	96.6	323	681
1.0%-B	76.0	141	2.35	149	274	117	391	759
1.0%-C	93.4	148	1.90	195	214	94.8	309	747
3.0%-A	66.3	111	1.95	50.3	200	78.9	279	508
3.0%-B	87.1	132	2.65	57.9	247	104	351	631
3.0%-C	87.1	164	3.40	146	267	101	368	769
5.0%-A	68.8	112	2.95	32.8	224	90.2	314	531
5.0%-B	87.0	177	3.50	129	234	99.8	334	730
5.0%-C	70.0	114	6.30	64.6	186	77.9	264	519
8.0%-A	75.4	124	0.38	47.6	220	97.8	318	565
8.0%-B	85.4	133	8.05	58.1	194	76.4	270	555
8.0%-C	84.2	145	2.90	11.9	202	93.1	295	539

Table B-4. Comparison of percent desorbed for surfactants studied: Total Explosives.

Surfactant Weight Percentage	Tween 80		Witconol		Simple Green	
	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation
0	41	3	43	4	44	0
0.1	45	5	43	2	45	2
0.5	35	5	41	3	47	2
1	51	11	55	4	40	1
3	65	8	68	2	40	2
5	67	7	73	1	44	5
8	65	6	75	6	46	2
10	74	2	58	16	N/A	N/A
15	71	15	68	12	N/A	N/A
20	80	4	72	1	N/A	N/A

Table B-5. Comparison of percent desorbed for surfactants studied: TNT.

Surfactant Weight Percentage	Tween 80		Witconol		Simple Green	
	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation
0	42	2	40	5	37	3
0.1	35	0	39	4	43	3
0.5	35	4	31	17	48	3
1	68	21	52	4	45	3
3	53	14	53	6	45	1
5	57	9	72	1	40	14
8	53	11	64	12	29	14
10	64	4	49	23	N/A	N/A
15	73	21	59	17	N/A	N/A
20	80	5	64	8	N/A	N/A

Table B-6. Comparison of percent desorbed for surfactants studied: HMX.

Surfactant Weight Percentage	Tween 80		Witconol		Simple Green	
	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation
0	22	2	31	12	37	6
0.1	24	5	35	13	34	3
0.5	28	3	29	10	30	6
1	37	5	45	10	29	5
3	66	21	64	4	36	4
5	61	4	67	3	39	5
8	66	4	73	6	36	5
10	65	4	47	17	N/A	N/A
15	65	19	57	8	N/A	N/A
20	79	5	62	3	N/A	N/A

Table B-7. Comparison of percent desorbed for surfactants studied: RDX.

Surfactant Weight Percentage	Tween 80		Witconol		Simple Green	
	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation
0	63	6	66	3	69	1
0.1	64	3	67	1	66	2
0.5	58	4	62	3	66	4
1	62	9	68	4	57	3
3	73	5	78	2	57	2
5	76	6	80	0	58	7
8	75	3	80	6	55	6
10	82	1	75	7	N/A	N/A
15	78	11	78	6	N/A	N/A
20	86	2	81	2	N/A	N/A

Table B-8. Comparison of percent desorbed for surfactants studied: Amino DNT.

Surfactant Weight Percentage	Tween 80		Witconol		Simple Green	
	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation	% Desorbed	Standard Deviation
0	30	3	30	3	23	1
0.1	39	16	31	2	25	1
0.5	19	14	33	5	28	2
1	34	8	50	4	22	2
3	62	8	66	2	22	4
5	65	10	70	3	30	3
8	62	6	73	5	44	3
10	71	2	49	22	N/A	N/A
15	66	17	64	17	N/A	N/A
20	75	7	70	0	N/A	N/A

Appendix C

Sequential Desorption Data

Table C-1. Sequential batch desorption: Yorktown soil and DDI water.

Sample	HMX	RDX	TNT	4A-DNT	2A-DNT	Total Explosives
0%-A1	3.35	43.1	10.3	21.4	7.48	85.63
0%-B1	3.96	48.9	5.38	21.4	7.29	86.93
0%-C1	3.4	54.8	6.48	23	7.91	95.59
0%-A2	5.56	7.16	1.65	7.28	2.81	24.46
0%-B2	3.65	8.27	1.01	7.43	2.78	23.14
0%-C2	4.95	9.88	1.17	8.12	3.1	27.22
0%-A3	3.9	1.09	0.406	2.92	1.21	9.526
0%-B3	4.6	1.14	0.23	2.82	1.16	9.95
0%-C3	5.47	1.321	0.244	2.77	1.17	10.975
0%-A4	1.17	0.278	0.079	1.18	0.495	3.202
0%-B4	0.931	0.203	0.115	1.14	0.487	2.876
0%-C4	1.67	0.297	0.096	1.25	0.54	3.853
0%-A4S	2.2	5.11	11.8	65.6	51.7	136.41
0%-B4S	16.7	26.4	24.6	159	99.3	326
0%-C4S	8.5	100	15.5	92	70	286

Soil Samples denoted by an S on the end of the I.D. number. Units are mg/l for Aqueous samples and mg/kg for soil samples

Table C-2. Sequential batch desorption: Yorktown soil and 3% Tween 80.

Sample	HMX	RDX	TNT	4A-DNT	2A-DNT	Total Explosives
3%-A1	5.31	51	66.4	51.9	14.3	188.91
3%-B1	4.4	35	38.7	45.3	12.2	135.6
3%-C1	5.12	40	46.7	36.4	11.2	139.42
3%-A2	10.4	30.6	19.2	18.4	6.6	85.2
3%-B2	3.23	21.1	16	19.8	5.78	65.91
3%-C2	8.9	25.9	22.5	17.4	6.49	81.19
3%-A3	3.54	7.07	3.31	6.26	2.29	22.47
3%-B3	5.25	16.4	5.08	12.8	4.22	43.75
3%-C3	6.97	8.56	5.97	9.89	4.38	35.77
3%-A4	1.88	1.82	1.03	2.76	1.15	8.64
3%-B4	7.76	5.12	1.94	5.34	2.17	22.33
3%-C4	4.74	2.55	2.38	4.6	2.53	16.8
3%-A4S	43.9	22	40.7	68.6	56.7	231.9
3%-B4S	42.2	8.14	18.4	31.1	28.9	128.74
3%-C4S	19.3	27.2	40.5	81.5	61.8	230.3

Soil Samples denoted by an S on the end of the I.D. number. Units are mg/l for Aqueous samples and mg/kg for soil samples

Table C-3. Sequential batch desorption: Yorktown soil and 5% Tween 80.

Sample	HMX	RDX	TNT	4A-DNT	2A-DNT	Total Explosives
5%-A1	5.5	43.8	62.2	44.2	15.4	171.1
5%-B1	3.33	29.6	53.3	34	11.7	131.93
5%-C1	3.08	31.4	46.4	34.9	0.9	116.68
5%-A2	10.6	19.7	15.4	14.7	6.4	66.8
5%-B2	3.15	18.4	27.6	15.3	6.4	70.85
5%-C2	7.37	23.7	18.9	15.5	0.3	65.77
5%-A3	6.15	5.04	3.07	5.44	2.91	22.61
5%-B3	2.32	6.74	7.38	5.73	2.82	24.99
5%-C3	4.82	6.05	3.24	5.88	0.18	20.17
5%-A4	1.65	1.02	0.83	1.73	0.97	6.2
5%-B4	2.07	3.83	10	3.97	2.5	22.37
5%-C4	1.27	1.15	0.89	1.74	0.08	5.13
5%-A4S	10.2	9.42	16.9	37.1	38.8	112.42
5%-B4S	50.1	8.88	16.7	14.9	11	101.58
5%-C4S	18.8	21.6	24	47.4	43.7	155.5

Soil Samples denoted by an S on the end of the I.D. number. Units are mg/l for Aqueous samples and mg/kg for soil samples

Table C-4. Sequential batch desorption: Yorktown soil and 3% Witconol SN 120.

Sample	HMX	RDX	TNT	4A-DNT	2A-DNT	Total Explosives
3%-A1	16.6	72.6	8.43	56	16.1	169.73
3%-B1	16.6	79	21.5	59.9	18.1	195.1
3%-C1	15.7	71.2	9.39	67	17.1	180.39
3%-A2	6.54	10.4	0.33	13.3	4.12	34.69
3%-B2	7.72	14.5	0.479	17.9	5.12	45.719
3%-C2	7.29	10.9	0.076	15.6	4.35	38.216
3%-A3	3.54	7.07	3.31	6.26	2.29	22.47
3%-B3	4.73	2.53	0.107	5.25	1.67	14.287
3%-C3	3.26	0.93	0.023	4.26	1.35	9.823
3%-A4	0.53	0.17	0.06	0.6	0.23	1.59
3%-B4	0.648	0.27	0.12	0.79	0.27	2.098
3%-C4	0.469	0.115	0.03	0.63	0.217	1.461
3%-A4S	22.5	8	98	27.5	22.5	178.5
3%-B4S	15.8	10.8	24.2	38.7	29.2	118.7
3%-C4S	11.4	2.88	8.01	18.4	12.2	52.89

Soil Samples denoted by an S on the end of the I.D. number. Units are mg/l for Aqueous samples and mg/kg for soil samples

Table C-5. Sequential batch desorption: Yorktown soil and 5% Witconol SN 120.

Sample	HMX	RDX	TNT	4A-DNT	2A-DNT	Total Explosives
5%-A1	18	69.6	11.7	58.3	15.4	173
5%-B1	19.9	78.6	14.7	66	11.7	190.9
5%-C1	18.2	73.6	18.7	59.7	15.2	185.4
5%-A2	5.32	14.3	2.64	12.1	3.41	37.77
5%-B2	7.03	13.5	1.25	13.7	3.61	39.09
5%-C2	7.7	12.2	0.669	12.7	3.73	36.999
5%-A3	4.34	1.75	0.023	3.42	1.07	10.603
5%-B3	4.08	1.81	0.067	3.37	0.98	10.307
5%-C3	3.34	1.59	0.08	3.04	0.94	8.99
5%-A4	0.494	0.163	0.06	0.6	0.2	1.517
5%-B4	2.07	3.83	10	0.471	2.5	18.871
5%-C4	0.335	0.139	0.04	0.471	0.15	1.135
5%-A4S	20.6	8.26	14.9	27.6	20.9	92.26
5%-B4S	50.1	8.88	16.7	14.9	11	101.58
5%-C4S	8.18	4.22	9.86	21	17.9	61.16

Soil Samples denoted by an S on the end of the I.D. number. Units are mg/l for Aqueous samples and mg/kg for soil samples

Appendix D

Desorption Kinetics Data

Table D-1. Desorption kinetics raw data. Concentration in the aqueous phase in milligrams per liter.

Time (hrs)	HMX DDI Water	HMX 3% Tween	RDX DDI Water	RDX 3% Tween	TNT DDI Water	TNT 3% Tween
0.50	0.46	0.35	5.96	4.98	50.20	75.50
1.00	0.70	0.43	9.09	6.84	59.20	73.80
2.00	0.98	0.77	12.00	11.20	62.80	75.70
6.00	1.51	1.63	14.50	14.60	72.60	101.00
12.00	1.96	2.37	15.20	13.90	81.60	138.00
24.00	2.24	3.33	15.40	13.60	83.40	173.00
48.00	2.17	3.73	14.30	12.40	73.60	179.00
96.00	2.16	3.57	12.80	11.40	64.40	126.00

Table D-1 (Continued). Desorption kinetics raw data. Concentration in the aqueous phase in milligrams per liter.

Time (hrs)	4A-DNT DDI Water	4A-DNT 3% Tween	2A-DNT DDI Water	2A-DNT 3% Tween	Explosives DDI Water	Explosives 3% Tween
0.50	3.91	7.45	1.69	3.29	62.22	91.57
1.00	4.66	7.71	1.89	3.38	75.54	92.16
2.00	4.73	7.99	1.94	3.36	82.45	99.02
6.00	5.00	8.62	2.07	3.85	95.68	129.70
12.00	5.42	9.27	2.26	4.45	106.44	167.99
24.00	5.31	9.37	2.30	5.19	108.65	204.49
48.00	4.92	9.03	2.27	6.39	97.26	210.55
96.00	4.10	8.47	2.34	7.02	85.80	156.46

APPENDIX C
TWO ENGINEERED APPROACHES FOR TREATMENT OF EXPLOSIVES-CONTAMINATED SOILS USING BOTH AEROBIC AND ANAEROBIC CONSORTIA (DRAFT)

NOTE: **The attached report is the author's review draft. Per the author's request, the report should not be cited. A final report will be included once available.**

Technical Report
September 1996

US Army Corps
of Engineers
Waterways Experiment
Station

TWO ENGINEERED APPROACHES FOR TREATMENT OF EXPLOSIVES-CONTAMINATED SOILS USING BOTH AEROBIC AND ANAEROBIC CONSORTIA

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Strategic Environmental Research Development Program

Technical Report
September 1996

TWO ENGINEERED APPROACHES FOR TREATMENT OF EXPLOSIVES-CONTAMINATED SOILS USING BOTH AEROBIC AND ANAEROBIC CONSORTIA

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Final report of a series

Prepared for U.S. Army Corps of Engineers
Washington DC 20314-1000

Under

Monitored by Environmental Laboratory
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WES Diagram

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Preface

The work reported herein was conducted by the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, as part of remediation technology assessments by the Naval Facilities Engineering Command (NFEC), Atlantic Division 1824, Norfolk, Virginia. Additionally, this work was also part of the Strategic Environmental Research and Development Program (SERDP), Installation Restoration Research Program (IRRP) and the U.S. Army Environmental Quality Technology Research Program.

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This report should be cited as follows:

> Harvey et al. (1996). "Two Engineered Approaches for Treatment of Explosives-Contaminated Soils Using both Aerobic and Anaerobic Consortia", Technical Report, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

1.0 INTRODUCTION

Explosives contaminated soils are a widespread problem in the Department of Defense. The source of these contaminated soils is usually from past manufacturing and/or weapons assembling operations. It is reported by DoD that there are approximately 540,000 cubic meters (700,000 cubic yards) of explosives contaminated soil located on DoD facilities that will require cleanup (1). Current technology for treatment of these soils is incineration which tends to cost within the range of $350 - $1,200 per cubic yard. Also, siting of an incinerator has become both a regulatory and publicity nightmare. Clearly, a more cost effective and better recieved technology needs development to assist DoD with the remediation of its many explosives contaminated sites. The primary explosives of interest is 2,4,6-trinitrotoluene (TNT) due to its widespread usage by the US Military since the turn of the century. Other explosives of note include hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX).

The Naval Weapons Station Yorktown, Yorktown, Virginia (WPNSTA Yorktown) is a 10,624 acre installation located on the Virginia Peninsula in York and James City Counties and the City of Newport News. The WPNSTA Yorktown is located approximately 10 miles north of Newport News, Virginia. The primary mission of WPNSTA Yorktown is to provide ordnance, technical support, and related services to sustain the war-fighting capability of the armed forces in support of national military strategy. WPNSTA Yorktown was established in 1918 to support mine laying during World War I. During World War II, the facility was expanded by the addition of three trinitrotoluene (TNT) loading plants. These past military activities associated with weapons loading and packing have resulted in the contamination of surface soils with explosives compounds such as 2,4,6-trinitrotoluene (TNT), hexahydrotrinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX).

Numerous investigators have studied explosives degradation within biological systems (McCormick et al. 1976, Carpenter et al. 1978, Kaplan and Kaplan 1982, Fernando et al. 1990, Funk et al. 1993, Boopathy et al. 1993, Gilcrease and Murphy 1996). These studies have been under aerobic conditions (McCormick et al. 1976, Funk et al. 1993, Manning et al. 1995) or anaerobic conditions (McCormick et al. 1976, McCormick et al. 1978). Most studies utilized native soil bacterial consortia; however, some efforts have focused on the use of specific isolates (McCormick et al. 1976, McCormick et al. 1978,Kaplan and Kaplan 1982, Boopathy et al. 1993, Grigsby et al. 1996). A variety of reactor systems have been evaluated for use with these biological systems (Funk et al. 1993, Zappi et al. 1993, Manning et al. 1995). Degradation pathways have been proposed for both aerobic and anaerobic conditions (Won et al. 1974, McCormick et al. 1976, Spain et al. 1995). In general, most biological systems studied indicate that the stepwise reduction of the nitro-groups appears to be the most common degradation path. This pathway results in the formation of amino-substituted by-products such as amino-dinitrotoluenes, diamino-nitrotoluenes, and triaminotoluenes (Won et al. 1974, McCormick et al. 1976, Spain et al. 1995).

Composting experiments have indicated that under aerobic conditions, TNT and related by-products can bind irreversibly to organic matter (Kaplan and Kaplan 1982, Pennington et al. 1995). Over 50 % of the added radiolabeled TNT was found to be irreversibly bound in an aerobic compost system (Pennington et al. 1995). Only under anaerobic conditions has significant CO_2 production been observed (Carpenter et al. 1978). Recent studies indicate that both bioslurry and bioagricultural reactors can be successfully used in the remediation of explosives contaminated soil under aerobic conditions (Zappi et al. 1993). As a point of note, the bioagricultural experiments did not indicate the formation of azoxytoluenes within the biologically active cells, unfortunately, the HPLC standard for this by-product was not available for the bioslurry experimentation phase (Carpenter et al. 1978). One study indicates an impressive increase in explosives and by-product removal rates accomplished through the addition of a non-ionic surfactant (Zappi et al. 1993).

The status of the environment at WPNSTA Yorktown is being investigated through the Department of Defence's Installation Restoration Program (IRP). In October 1992, WPNSTA Yorktown was finalized for inclusion on the National Priorities List (NPL).

In support of the US Naval Facilities Engineering Command, the USAE Waterways Experiment Station (WES) evaluated several biological processes to treat explosives contaminated soil. These processes included aerated biotreatment, anaerobic biotreatment, and the addition of exogenuous organisms to stimulate biodegradation. These experiments were conducted in biocell and bioslurry bench reactors to ascertain the advantages of mixing. Additionally, the enhancement of explosives desorption from soils by non-ionic surfactants was evaluated.

BACKGROUND

Biotreatment processes are destruction processes that utilize enzymatic mechanisms catalyzed by microorganisms to break-down organic compounds. Biotreatment processes have been widely used for treatment of municipal and industrial wastewater and groundwater treatment. Recent developments in both bioreactor design and microbiology have resulted in the use of biotreatment processes for remediation of contaminated solids (soils, sediments, and sludges). This study investigated two biotreatment approaches, aerobic and anaerobic, using two application scenarios, bioslurry and biocell reactors.

Aerobic microorganisms

Aerobic microorganisms require oxygen as a terminal electron acceptor during respiration and for biosynthesis of fatty acids. Organisms utilizing organic compounds as electron donors are referred to as heterotrophs, while those obtaining all of their energy from sources other than organic compounds are termed autotrophs. Many bacteria, and

most fungi, algae, and protozoa are obligate aerobes (i.e. they require oxygen for growth). Lack of sufficient levels of oxygen in a medium can often be responsible for poor growth of aerobic microorganisms.

Anaerobic microorganisms

Anaerobic microorganisms utilize biochemical reactions wherein oxidized compounds serve as electron acceptors and are reduced. This process is fueled by the oxidation of organic or inorganic compounds. Once almost all dissolved oxygen has been utilized, facultatively anaerobic bacteria, capable of growth in both aerobic and anaerobic environments, take over from aerobic microorganisms, and other electron acceptors are used in place of oxygen. Initially, nitrate is reduced. Upon consumption of all nitrate supplies, manganese IV is reduced, followed by iron III, sulfate, and then carbon dioxide. Most obligate anaerobes use organic materials to produce carbon dioxide and methane, and some are extremely intolerant of oxygen.

TNT Biodegradation

Biological degradation of an explosive to form its basic inorganic components (carbon dioxide, water, and nitrate in the case of nitro-aromatics) is termed mineralization. Measurable losses of an explosive, such as TNT, from contaminated media is termed degradation. If the mechanisms of degradation are biological in nature, then the term "biodegradation" is often used. Degradation of TNT does not necessarily indicate that mineralization or even aromatic ring cleavage has occurred. Without complete mineralization occurring, intermediates (by-products) of TNT degradation may still be present. To date, a microbial pathway responsible for complete mineralization of TNT using aerobic consortia has not been fully demonstrated.

Earlier work by several investigators indicated that TNT can be biologically transformed into several by-products, some of which are more toxic than the parent TNT molecule (Carpenter, McCormick, Cornell, and Kaplan 1978; McCormick, Feeherry, and Levinson 1976; Kaplan and Kaplan 1982, Parrish, 1977). The reduction of the nitro groups proceeds through the nitroso and hydroxylamino compounds. All products resulting from TNT reduction (amino, diamino, and azoxy compounds) originate from the hydroxyamino compound (McCormick et al. 1976).

Won et al. (1974) used shake flasks to show that TNT was cometabolically transformed under aerobic conditions by *Pseudomonas* sp Y into the following products: 2-amino-4,6-dinitrotoluene (2A-DNT), 4-amino-2,6-dinitrotoluene (4A-DNT), 2,6-dinitro-4-hydroxylaminotoluene, diaminonitrotoluene, and various azoxytoluene complexes. Won et al. postulated that the formation of azoxy compounds was due to coupling reactions - not metabolism.

Carpenter et al (1978) examined the fate of ^{14}C-labeled TNT in an activated-sludge system. After 3-5 days, neither ^{14}C-TNT nor ^{14}C-CO_2 was detected. After centrifuging,

the distribution of ^{14}C was equally divided between the supernatant and floc material. Within the floc, 30% of the 50% total ^{14}C was found in the lipid fraction. An insoluble material was found within the lipid fraction that the authors determined to be polymers formed from a reaction between cell components and TNT reduction products.

Kaplan and Kaplan examined the fate of ^{14}C-labeled TNT in a compost system and found that TNT was transformed to amino-dinitrotoluene, diamino-nitrotoluene, and azoxytoluene complexes. No ^{14}C-CO_2 was recovered and found that a significant portion of radiolabeled material was bound to the humic fraction of the compost.

McCormick et al. (1976) used anaerobic conditions generated by a hydrogen atmosphere and enzyme preparations of V. alalescens to reduce TNT to amino, diamino, and triamino-nitrotoluene products. The authors stated that the reducing potential of the system determined whether all three nitro groups are reduced and that azoxytoluene complexes were formed by the nonenzymatic oxidation of hydroxylamino-dinitrotoluene compounds.

Bradley et al. (1994) investigated the ability of using native consortia from surface soils and aquifer materials to degrade TNT. The authors used ^{14}C-labeled TNT in batch shake flasks under aerobic conditions. Aqueous phase TNT concentration was removed between 20 to 70 days. TNT was reduced through the amino and diamino intermediates and approximately 10% of the ^{14}C-TNT was mineralized to ^{14}C-CO_2 within 35 days.

Evidence has been presented by researchers from the University of Idaho for definition of an anaerobic pathway for TNT mineralization (presentation made by Crawford and Crawford 1994). Additionally, McCormick et al. (1976) showed production of triaminotoluene (TAT) from TNT under anaerobic conditions.

Parrish (1974) examined the ability of 190 fungi representing 98 genera to transform TNT under aerobic conditions. Parrish found that fungi could commonly transform only one nitro group into products such as amino, hydroxyamino, and azoxy compounds. Parrish found the p-nitro group to be preferentially reduced. Based on these results, and the inability of the fungi to transform 2,4 DNT, Parrish suggested that application of fungi to degrade TNT and DNT contaminated wastewaters was not promising.

Kaplan and Kaplan (1982) identified the following degradation by-products: 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2',4,6',6-tetranitro-2,4'-azoxytoluene, 2,2',6,6'-tetranitro-4,4'-azoxytoluene. They also proposed a biotransformation scheme which included three products, 4-hydroxylamino-2,6-dinitrotoluene, 2-hydroxylamino-4,6-dinitrotoluene, and 4,4'-6,6'-tetranitro-2,2'-azoxytoluene (however, they were unable to identify these compounds in their extracts). The microorganisms active in the degradation of TNT were not identified during their studies; however, these studies were performed under aerobic conditions.

Recent work by the researchers at the University of Idaho (personnel communication with Dr. Don Crawford 1994; personnel communication with Bill Doeksen, 1994) indicates that an anaerobic pathway for degradation of TNT to organic acids has been identified. The proposed pathway requires complete aminozation of the TNT molecule to triaminotoluene (TAT) followed by conversion of TAT to p-cresol. The pathways through TAT is similar to a anaerobic pathway used by a plant based reductase that has been recently proposed by researchers at the USEPA's Athens Laboratory (personal communication with Dr. Steve McCutcheon 1994). Ring cleavage of the cresol results in the formation of several organic acids which are resistive to further degradation by anaerobes. However, when aerobic conditions are established, the organic acids are mineralized to carbon dioxide and cell mass.

RDX and HMX Biodegradation

Degradation of RDX in sediments and mineralization to CO_2 under anaerobic conditions was demonstrated by Spanggord et al. (1980) and Sikka et al. (1980), respectively. However, these researchers were unable to identify the products of degradation.

McCormick, Cornell, and Kaplan (1981) identified the products of microbial degradation to include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, formaldehyde, and methanol. Some of these products may be carcinogenic and/or mutagenic, especially the N-nitroso compounds. McCormick, Cornell, and Kaplan (1981) also proposed a biodegradation scheme which accounted for all of the observed products and proceeds through the successive reduction of nitro groups until destabilization and ring fragmentation occur. In this scheme RDX is sequentially reduced to the nitroso derivatives of MNX, DNX, and TNX. McCormick, Cornell, and Kaplan demonstrated conclusively that biodegradation proceeds only under anaerobic conditions. The authors suggest that remediation efforts include an initial anaerobic step to reduce RDX wastes to hydrazines and methanol, followed by an aerobic step to oxidize the methanol.

Surfactant-Enhanced Biodegradation

Some factors governing the availability of contaminants to microorganisms in a bioslurry reactor are not well understood. However, factors known to exert prominent influence include the aqueous solubility of the contaminant and the rate of diffusion/mass transfer of the contaminant from soil solids to the aqueous phase. Aqueous solubility and mass transfer can be increased by addition of a surfactant which lowers the surface tension of the soil/water slurry and at sufficient concentrations forms micelles which act as another phase into which contaminants can partition. Surfactants have been used to enhance recovery of gasoline in groundwater (Texas Research Institute 1980) and as dispersants for petroleum spills (BioSafe Inc. 1989). Based on the positive aspects of surfactant addition in other biotreatment studies, the feasibility of surfactants were evaluated as part

of this study. Surfactants selected for evaluation in this study are considered "green" surfactants which, by definition, are considered safe for application in human food processing activities by the US Food and Drug Administration (Personal communication with Microenvironmental Inc. 1991). These surfactants are generally considered safe in terms of compatibility with microorganisms (Personal communication with MicroEnvironmental Inc. 1991 and PPG-Maiser Inc. 1990).

TWO ENGINEERED APPROACHES TO BIOTREATMENT

Two biotreatment application scenarios were evaluated during this study. They essentially differ from each other in terms of the level of mixing obtained within each system. Bioslurry represents the highest level of mixing available, while biocells are static systems. Mixing represents one of the most costly portions of process unit costs. As such, the rationale for evaluation of two reactor configurations is the potentially dramatic differences in treatment costs that may be realized by WPNSTA Yorktown. Bioslurry systems are estimated to cost between $90 - $200 per cubic yard treated depending on the removal kinetics obtained and the amendment doses required. Biocells are estimated to costs between $20 - $100 per cubic yard treated also depending on removal kinetics and amendment requirements.

Bioslurry Reactors

Bioslurry treatment of contaminated soils is a relatively new treatment technology for destruction of biodegradable contaminants sorbed to soil particles and/or in solution within the interstitial pore water. Bioslurry treatment is an engineering reconfiguration of other more widely used biotreatment technologies, such as land treatment and composting, that have been successfully used for decontamination of soils and sludges (Montemagno and Irvine 1990; Gunnison 1991).

Bioslurry treatment is similar to other soil and sludge biotreatment technologies in terms of microbiological interactions and contaminant degradation pathways. However, it differs from the other technologies, because bioslurry systems are capable of substantially increasing the degradation rate of contaminants by increasing the availability of contaminants, electron acceptors, nutrients, and other additives to the microbial consortia. This is accomplished by completely mixing the soil in a water slurry (typically at 30 percent solids); thereby, reducing mass transfer limitations associated with the biotreatment of soils contaminated with hydrophobic contaminants having high sorption coefficients. For aerobic systems, oxygen levels are maintained by diffusion of air or oxygen into the soil/water slurry. The result of these operational features is a biological system that is conducive to optimal microbial activity and increased contaminant degradation rates (Zappi et al. 1991).

Figure 1-2 is a photograph of the bench-scale bioslurry reactors used in this study. These units were designed to simulate commercially available pilot and full scale bioslurry reactors. The reactors have a 5 liter working volume and are constructed of glass. The

reactor has two sampling ports located at the middle and bottom. Three probe ports are located on the sides of the reactor for monitoring pH and ORP. Three diffuser ports for aerobic conditions are located on the bottom. The reactors used 1/15 hp mixers set at 200 rpm to mix the soil slurry. Two impellers were used to mimic commercial bioslurry reactor mixing kinetics. A directional propeller was located on the shaft bottom; this forced the slurry downward to the circular bottom then upward along the reactor walls. As the slurry was forced along the reactor walls, the paddle mixer mounted on the same shaft caused a lateral mixing action that forced the solids to be rotated around the reactor walls. Mixing indents (baffles) along the reactor walls was used to force the slurry inward toward the middle of the reactor, before moving downward to the reactor bottom.

Figure 1-3 illustrates a conceptual approach to implementing bioslurry systems in the field. Field screening of the untreated soils is often required to remove large debris and gravel from the soils prior to bioslurry treatment. Bioslurry systems are typically operated in the batch or semi-batch mode.There are a variety of dewatering systems that may be used to effectively dewater the treated soils such as sludge drying beds and filter presses. Most of these are commercially available from the wastewater treatment industry.

Biocell Reactors

Biocells are an economically attractive, biotreatment process design for remediation of contaminated soils. The technology involves the excavation of soil, screening to remove larger debris, and loading into the biocell. Biocells are best described as "bioventing in a can". Once the soil is loaded into the biocell, little mixing is provided.

Biocells are operated in a true batch mode much like composting. The soil is added into the biocell without slurrying like the bioslurry process. Instead, the soil is simply dumped into the cell and aeration initiated to stimulate the aerobes. In some cases, if the soil has a very low hydraulic conductivity, sand or other bulking agents may be added to increase the hydraulic conductivity. Low hydraulic conductivity hinders transport of air (which supplies the oxygen) and water (which supplies the moisture and amendments). Anaerobic conditions are achieved by placing a water head above the soil mass to be treated. The water serves as a barrier to oxygen - greatly reducing its concentration in the soil phase. Additionally, the addition of carbon stimulates aerobic microorganisms to consume all dissolved oxygen since it serves as the electron acceptor.

Figures 1-4 and 1-5 are photographs of the aerated and anaerobic bench scale biocell reactors used in this study. This unit was chosen as it mimics commercially available drums, canisters, and dumpsters. This reactor has approximately a 30 liter working volume. These reactors were periodically mixed using the same mixers described above. The anaerobic reactors were not modified. The aerobic reactors had an air diffuser placed on the bottom with pea gravel for support and drainage. Samples were taken by removing the lid and scooping portions from different locations at the bottom. ORP and pH were monitored by placing probes into the aqueous phase.

A WES developed biocell design, which is illustrated in Figure 1-6, involves the use of a fixed-wall cell in which soil is added into a cell. This design utilizes 40 cy garbage dumpsters as the biocell unit. The benefit of using the dumpsters are dramatically reduced capital costs, as dumpsters typically lease for approximately $90 per month and are universally available. The cell contains a drainage layer on the bottom for recirculation of water and amendments. Air lines are buried in the soil pile for maintenance of aerobic conditions if aerobic treatment is attempted.

Moisture within the soil pile is maintained by periodic irrigation using soaker hoses usually laid out on the top of the soil pile. Moisture meters are buried within the soil pile to determine when irrigation is required. The irrigation operation is an excellent mechanism for supplying key nutrients, nitrogen and phosphates, to the microbial consortia.

It must be pointed out that biocells have been mostly used for petroleum hydrocarbon treatment. WES is unaware of any attempt to utilize the technology for explosives treatment; however, recent WES bench scale studies using Hastings soils (explosive contaminated) indicate that this approach is feasible for explosives.

Both application scenarios represent various positive and negative aspects to the WPNSTA Yorktown study. Bioslurry systems offer the most rapid removal kinetics of any soils biotreatment process available. The use of bioslurry system at YNWS allows the design engineers an option that may very quickly remediate the soils under a high degree of process control. Unfortunately, the WPNSTA Yorktown will have to pay higher unit treatment costs for these positive aspects. On the other hand, biocells provide conditions that result in kinetically slower biodegradation; however, potentially high reductions in unit treatment costs could be realized if biocells are proven feasible. In summary, if both techniques are proven viable, then the design team will have significant options to exercise during development of the remediation plans.

KEY PAST BIOTREATMENT STUDIES

Various biotreatment studies pertinent to the approach proposed in this document are discussed below. The studies highlighted involve biotreatment of explosives contaminated soils as opposed to water, which was the primary research interest in many of the past studies reviewed by WES.

Aerobic Biotreatment

More work has been done to date with aerobic biotreatment approaches than anaerobic. Much of the work published in literature is not encouraging; however, several studies recently completed by the US Army (as of yet, not published) indicate a high degree of potential of using aerobic systems for treatment of explosives contaminated soils.

Montemagno and Irvine (1990) reported TNT removals as high as 68 percent from soils collected from the Joliet Army Ammunition Plant using bioslurry systems. Soil-to-water ratios of 10-20 percent by-weight were used in this study. Although, they were not successful in proving that complete mineralization had occurred. The highest level of carbon dioxide produced in their study was approximately 0.5 percent. This work was done using radiolabelled (^{14}C) TNT. Finally, Montemagno and Irvine speculated that increased soil-water ratios may be more conducive to increased biodegradation of TNT due to improved conditions within the higher soils loaded reactor that may promote increased TNT utilizer growth.

The WES has recently completed a study which investigated that use of aerobic bioslurry systems with surfactant amendments for treatment of explosives contaminated soils from the Hastings East Industrial Park, Hastings, Nebraska. This research indicated that the addition of a commercially available surfactant, Tween 80, dramatically increased the degradation rates of both TNT and its by-products. These studies were performed using 5 liter bench scale slurry reactors operated in batch mode. Acetate was used as a co-metabolite in this study. TNT was reduced from 18,000 mg/kg to less than 10 mg/kg within a 7 week period. Amino-dinitrotoluenes were found to have much slower removal kinetics than any of the other analytes. This observation indicates that they will likely be rate limiting during full scale operation. Over the past several years, WES has evaluated both native and exotic microbial consortia for explosives biodegradation. To date, the native consortia have generally performed as effectively as the seeded organisms (exotics). During one study, WES did observe slightly better removal kinetics using an exotic WES consortium; however, the soil used in this study had much lower microorganism populations than the test soils previously used in other studies.

During early 1994, WES evaluated the potential for using aerobic processes for treatment of TNT contaminated soils using an in-place approach. The in-place approach involves treatment of explosives contaminated surface soils without excavation. This approach was evaluated because much of the explosives contamination found at DoD sites are located within the surface regions of the soil profile. The bench units used by the WES were similar to the proposed biocell systems for the WPNSTA Yorktown study in that aeration was provided via forced aeration using buried spargers. Surfactant amendments applied to the WES in-place systems resulted in similar by-product formation but with slower removal kinetics. Surfactant addition resulted in more rapid removal kinetics than the systems not amended with the surfactant. This study only evaluated the native consortia.

The US Army Environmental Center and Argonne National Laboratory recently completed a bench study which used molasses as a co-metabolite. Succinate was originally used as a co-metabolite, but molasses was evaluated later in the study because it is much cheaper than succinate. The molasses appeared to perform as well as the succinate. The AEC study had similar removal kinetics to those observed in the WES bioslurry systems without surfactant addition during their study phase which used batch systems operated like the WES systems. However, this study also operated the bioslurry

reactors under three candidate semi-batch mode operational strategies which focus on rate of soil slurry exchange. Three loading rates were evaluated; 15 percent charged weekly, charged twice weekly, and charged three times weekly. The results indicated no build-up of reduced nitroaromatics (by-products) for the once and twice weekly charged systems; however the three times weekly charged systems did indicate a build-up of reduced nitroaromatics.

Anaerobic Biotreatment

The research team of Drs. Ron and Don Crawford at the University of Idaho have developed an anaerobic biotreatment process for remediation of nitroaromatics. The process was originally developed for use in remediation of dinoseb (a herbicide structurally similar to TNT) under funding provided by the J.R. Simplot Company (Simplot), Pocatello, Idaho. Over the past several years, the J.R. Simplot Company has obtained the commercial rights to the Idaho process and is now marketing the Idaho process as the SABRE process (Simplot Anaerobic Biological Remediation Ex-situ). Simplot has recently obtained exclusive world-wide licensing for the SABRE patent which was recently awarded to the Idaho Research Foundation.

The SABRE process involves the addition of a potato starch solution into a soil matrix usually in slurry form. The potato starch initiates a high rate of microbial activity which drives the reduction-oxidation potential within the soil matrix to anaerobic conditions (optimally at -200 mV). Under anaerobic conditions, the explosives are degraded to organic acids.

The SABRE process was evaluated by the USEPA's Superfund Innovative Technology Evaluation (SITE) Technology Program in January 1990 and 1992 for dinoseb and explosives, respectively. The explosives study was completed in late 1993. The preliminary results indicate that the SABRE process was effective in removing explosives compounds and their by-products using a biocell type reactor system. Unfortunately, this study did not analyze for the full suite of by-products (i.e. cresol and 2 amino-dinitrotoluene) that have been detected during degradation of explosives compounds during past bench studies.

The significance of these results is that semi-batch operated systems appear to provide systems that may be capable of sustaining much improved removal kinetics for the reduced nitroaromatics. The use of a commercial surfactant may significantly reduce the soil residence times within the bioreactors (potentially impacting unit costs in a very positive way). Also, the use of molasses appeared to be a viable option as a co-metabolite which can result in reduced process costs. In general, the past efforts performed by these researchers provide an excellent framework for the design of a technically sound treatability study. Finally, the anaerobic process appears to provide treatment mechanisms that are much better defined than the aerobic process. The combined use of the anaerobic process with surfactant addition may provide a system that affords the more rapid kinetics than any other system studied to date.

Determination of Degradation Kinetics - Elementary Reaction Rate Expressions

Typically, first order kinetics is assumed for the biological degradation of organic compounds. From a plot of the log concentration versus time the reaction coefficient (k) can be determined. The first order expression can then be used to predict the half-life of the contaminant in addition to the soil retention time in the reactor to reach a certain concentration. The time behavior of the concentration of the reactants in elementary reactions with simple orders is determined by integrating the rate law for a particular rate expression. The three most commonly used rate laws are zero, first, and second order reactions. To determine the exact reaction order for a given data set, the nth order expression can be used and solved for the unknown variable (n). These kinetic expressions will be used to determine the reaction order of the biological treatment processes, the half-life, and soil retention time. Provided below is the the integrated rate expression for each.

Zero Order: $$[A_t]_t = [A]_0 - kt$$

First Order: $$\ln \frac{[A]_t}{[A]_0} = -kt$$

Second Order: $$\frac{1}{[A]_t} = \frac{1}{[A]_0} + 2kt$$

nth Order: $$\frac{1}{[A]_t^{n-1}} - \frac{1}{[A]_0^{n-1}} = (n-1)kt$$

Experimental Design

The treatments selected for the biocell and bioslurry bench studies were based on the results of the radiolabled TNT studies (Evans et al. 1996). Table 1- depicts the conditions selected for treatment of the Yorktown soils in the bench scale bioslurry and biocell reactors. All conditions were replicated in two reactors except for the sterile controls in which only one reactor per condition was used. A total of 24 reactors were used (12-bioslurry and 12-biocell). The sterile controls consist of mercuric chloride addition in order to sterilize the soil. The purpose of the sterile control is to determine the significance of other treatments and abiotic processes. In some cases, treatments are replicated in both biocell and bioslurry reactors in order to differentiate the benefit of mixing.

Table 1-1 Treatment Conditions for Bioslurry and Biocell Reactors			
	Bioslurry		**Biocell**
Reactor #	**Aerobic**	Reactor #	**Aerobic**
1	Sterile Control	1	Sterile Control
2,3	No additives	2,3	Tw 80 & Molasses
4,5	Tw 80 & Molasses		**Anaerobic**
	Anaerobic	4,5	Potato Starch
6,7	Potato Starch	6,7	Tw 80 & Molasses
8,9	Simplot	8,9	Simplot
10,11	Simplot w/ 4hrs mixing	10,11	Molasses
12	Sterile Control	12	Sterile Control

Selection of Treatment Conditions Based on Results from ^{14}C-TNT Studies

The Tween 80 and Molasses treatment is a combination of these two treatments. Both Tween 80 and Molasses conditions resulted in mixed results in the ^{14}C-TNT shake flask study. Tween 80 resulted in good degradation in the bioslurry but not in the biocell. Molasses resulted in good degradation in the biocell but not in the bioslurry. Molasses has also proven to be a good cometabolite in remediation activities at Joliet Army Ammunition Plant, Illinois. The use of Tween 80 has been shown to reduce the soil residence time by half in prior research at WES. It is proposed that Tween 80 would make the explosives more available and the molasses would stimulate the microbes to rapidly reduce the explosives compounds.

Potato starch is the cometabolite for the Simplot process. Potato starch resulted in good results in the anaerobic study and is a relatively available and cheap carbon source. The use of Potato starch will allow comparison between the addition of exogenuous organisms (Simplot) and native consortia.

The Joliet process had promising results, however, the process would require the shipment of Joliet microorganisms which would be unrealistic on a large scale. Thus, molasses was chosen as a substrate due to its success at Joliet AAP and it is also inexpensive and readily available.

2.0 Materials and Methods

Soil Preparation Methods

The soil used for all studies was a composite sample from three different sites at the Naval Weapons Station at Yorktown, Virginia. The soil was homogenized and mixed prior to shipment to the Waterways Experiment Station. The 55-gallon drums were stored in the Hazardous waste research center (HWRC) cooler at 4 degrees Celsius (^{o}C). Soil was further homogenized by mixing and sieving. Soil was sieved using a U.S. Standard #4 sieve (4.76 mm opening). The sieve removed gravel, rocks, twigs, and other debris. The homogenized soil was subsequently analyzed for explosive compounds such as HMX, RDX, TNB, DNB, TNT, 4A-DNT, 2A-DNT, 2,6-DNT, and 2,4-DNT using EPA method 8330.

Biocell Loading

Containers of explosives contaminated soil were removed from the cooler and 13.6 kilograms (30lbs) of Yorktown soil was loaded into each biocell reactor. In the anaerobic reactors, 4.35 g of ammonium phosphate (NH_4PO_4) and 13.6 liters of phosphate buffer were added to provide nutrients for the native consortia. The buffer solution served two purposes: to control pH as the native consortia produced organic acids in anaerobic conditions and to act as a barrier to oxygen to keep the system anaerobic. The system was mixed with a Lightnin mixer until the slurry was homogeneous. The biocells were sealed with a removable lid and were impervious to light.

In the aerobic reactors, nutrients and the carbon source were diluted in DDI water and mixed into the soil. The soil was placed into the reactors with no amendments. Air was introduced into the biocell through a diffuser located at the bottom of the reactor. The diffuser was covered with pea gravel and a membrane to assure the distribution of air.

Bioslurry Loading

Approximately 1340 g of Yorktown soil was weighed out for each reactor. The soil was then placed into the bioslurry reactors and phosphate buffer was added to bring the volume to 5 liters which resulted in a 30% (wt/wt) slurry. Nutrients and the carbon source were added to the mixture based on the specified treatment conditions. The bioslurry reactors were mixed at 200 rpm and wrapped in foil to prevent photodegradation. Anaerobic reactors had nitrogen purged into the head space of the reactor. Aerated reactors had air sparged at the bottom of the reactor at a flow rate of 2078 ml/min. Aerobic reactors had oxygen sparged at a flow rate of 150 ml/min.

Amendments

The amendments are provided in table 5.? for both all conditions examined. The amendments included:

Tween 80 and Molasses - 3% wt/wt Tween 80 and 0.3% vol/vol molasses
Molasses - 0.3% vol/vol molasses
No Additives - 10 mg/l phosphorus and 30 mg/l nitrogen
Simplot - consortia and potato starch were added according to Simplot protocal
Potato Starch - addition followed Simplot protocal but did not include consortia
Sterile Controls - 1.5% wt/wt addition of $HgCl_2$

Dissolved Oxygen Analysis

Dissolved oxygen in the aerobic bioslurry reactors was analyzed using an Orion 840 Dissolved Oxygen meter and probe in conjunction with BOD sample bottles. The dissolved oxygen probe is a two-electrode system separated from the sample by an oxgyen permeable membrane. When a polarizing voltage is imposed across the system, it reduces dissolved oxygen a the cathode causing a measureable current to flow. The current varies directly with the diffusion of dissolved oxygen through the membrane, which is proportional to the partial pressure of oxygen outside the membrane. The instrument was calibrated daily and automatically corrects reading for temperature effects. Soil slurry sample was placed into a 300 ml BOD bottle and the initial reading at equilibration was recorded as the dissoved oxygen concentration.

pH Analysis

The pH was measured using a Beckman portable pH/mv meter with research grade pH and ORP electrodes. The pH electrode is a glass
bulb containing a fixed hydrogen chloride concentration in contact with an internal reference electrode. Upon immersion into solution, the bulb surface becomes hydrated and sodium ions are exchanged for hydrogen ions. Anions are repulsed by fixed, negatively charged silica sites which causes a potential at the glass-solution interface which is a function of the hydrogen ion activity in solution [Standard Methods]. A two point calibration with pH buffers of 4 and 7 was used to calibrate the pH probes on a weekly basis. The ORP probes were checked by using saturating pH buffer soluions (4 and 7) with quinhydrone to determine if the mv readings were in the appropriate range (pH4 240-280 mv; pH7 70-110 mv).

Ammonia Analysis

Bioslurry phase ammonia was analyzed using an Orion 901 ionanalyzer and an ammonia ion specific probe. The probe was calibrated using a five point calibration with ammonia standards of: 1mg/l, 10mg/l, 100 mg/l, 250 mg/l, and 1000 mg/l. Soil slurry sample was centrifuged and filtered prior to analysis. Fifty milliliters of sample was then placed into beaker with 1ml ISA solution (basic solution to drive dissolved ammonia to

gaseous phase) and mixed. An ammonia probe was placed into the head space and the reading taken at equilibration.

Oxygen Uptake Rate (OUR) Analysis

Oxygen uptake rate (OUR) was performed with an ORION 840 Dissolved Oxygen meter and probe as previously discussed. The probe was inserted into a 300 ml BOD bottle completely filled with soil slurry from the bioslurry reactors and readings were recorded every 5 minutes over a 30 minute interval. The change in dissolved oxygen with respect to time served as the oxygen uptake rate (hr^{-1}).

Temperature Analysis

The ambient temperature in the laboratory was measured using a hand held Fisher thermometer with a -10 to 200 °C range. The temperature in the bioslurry laboratory was maintained at approximately 25 °C and biocells were maintained between 19 to 28 °C.

Explosives Analysis

Explosive compounds were analyzed with a Waters High Performance Liquid Chromatograph (HPLC) consisting of a Waters 717 Autosampler, Waters 486 tunable aborobents detector, set at a wavelength of 245 nm, with a Supelco C-18 reverse phase primary column and a confirmed with a C-CN reverse phase column on a Waters LC module 1 at 245 nm. The mobile phase consisted of a 50/50 (v/v) methanol/water mixture at 1.2 ml per minute, with a run time of 21 minutes and injection volume of 100 ul.

Explosives were analyzed using EPA Method 8330. Soil slurry was centrifuged at 12,000 rpm in a Sorvall centrifuge. The supernatant was filtered through a 0.45 um filter. Both soil and aqeous phases were separated into 40 ml VOA vials and stored at 4C until analysis. Aqeous samples were mixed with methanol to form a 50/50 v/v mixture and analyzed.

Soil samples were air-dried and pulverized in an acetonitrile rinsed mortar. Two grams of dried sample are mixed with 10 ml acetonitrile in a 15 ml glass vial with teflon-lined cap, vortex swirled for one minute, and placed in an ultrasonic bath for 18 hours. After sonification, samples are allowed to settle for 30 minutes. Five mls of sample are combined with 5 mls of calcium chloride solution and shaken. Supernatant is filtered through a 0.5um filter and analyzed.

3.0 RESULTS

Biocell - Analysis of results by treatment

Molasses (Anaerobic)

Figure 3-1 is a plot of the average explosive compound concentrations versus time from the duplicate anaerobic Molasses biocell reactors and table A-1 is a summary of the explosive compound concentrations at the different sampling intervals. TNT is degraded from an average concentration of approximately 1069 mg/kg to less than 54 mg/kg in 84 days. The sum of 4A-DNT and 2A-DNT is initially at 242 mg/kg and remains above 200 mg/kg until day 21. The amino-DNT compounds are degraded to less than 12 mg/kg by day 70. The diamino-NT (DANT) compounds (2,4-DANT and 2,6-DANT) increase from 0 mg/kg to 112 mg/kg by day 21. The diamino-NT compounds are subsequently degraded to less than 4 mg/kg by day 70. The Molasses treatment showed no significant formation of the azoxy compounds with less than 3 mg/kg detected throughout the course of the experiment. RDX is degraded from 175 mg/kg to less than 11 mg/kg by day 70. HMX proves to be the most recalcitrant compound with an initial concentration of 90 mg/kg reduced to less than 20 mg/kg in 35 days. Overall, total explosives (the sum of all explosive compounds) is degraded from a concentration of 1577 mg/kg to less than 72 mg/kg in 84 days.

Tween 80 and Molasses Treatment (Anaerobic)

Figure 3-2 is a plot of the average explosive compound concentrations versus time from the duplicate anaerobic Tween 80 and Molasses reactors. TNT is degraded from an average concentration of approximately 1124 mg/kg to less than 20 mg/kg in 49 days . The sum of 4A-DNT and 2A-DNT is initially at 173 mg/kg and increases to 351 mg/kg by day 14. The amino-DNT compounds are degraded to less than 11 mg/kg by day 35. The diamino-NT compounds (2,4-DANT and 2,6-DANT) increase from 0 mg/kg to 102 mg/kg by day 21. The diamino-NT compounds are subsequently degraded to less than 4 mg/kg by day 84. The Tween 80 and Molasses treatment showed no significant formation of the azoxy compounds with less than 6 mg/kg detected throughout the course of the experiment. RDX is degraded from 165 mg/kg to less than 5 mg/kg by day 35. HMX was reduced from an initial concentration of 90 mg/kg reduced to less than 20 mg/kg by day 84. Overall, total explosives (the sum of all explosive compounds) is degraded from a concentration of 1560 mg/kg to less than 25 mg/kg in 84 days.

Simplot Treatment (Anaerobic)

Figure 3-3 is a plot of the average explosive compound concentrations versus time from the duplicate anaerobic Simplot reactors. TNT is degraded from an average concentration of approximately 909 mg/kg to less than 60 mg/kg in 84 days . The sum of 4A-DNT and 2A-DNT increased from 148 mg/kg to 480 mg/kg by day 14. The amino-DNT compounds were degraded to less than 10 mg/kg by day 84. The diamino-NT

compounds (2,4-DANT and 2,6-DANT) increased from 0 mg/kg to 293 mg/kg by day 21. The diamino-NT compounds are subsequently degraded to less than 10 mg/kg by day 49. The Simplot treatment showed no significant formation of the azoxy compounds with less than 1 mg/kg detected throughout the course of the experiment. RDX increases in concentration from 142 mg/kg to 323 mg/kg by day 14 and is subsequently degraded to less than 5 mg/kg by day 21. HMX was reduced from an initial concentration of 194 mg/kg to less than 10 mg/kg by day 84. Overall, total explosives (the sum of all explosive compounds) is degraded from a concentration of 1394 mg/kg to less than 75 mg/kg in 84 days.

Potato Starch Treatment (Anaerobic)

Figure 3-4 is a plot of the average explosive compound concentrations versus time from the duplicate anaerobic Potato Starch reactors. TNT is degraded from a maximum average concentration of 1305 mg/kg to less than 10 mg/kg in 84 days. The sum of 4A-DNT and 2A-DNT increased from 165 mg/kg to 306 mg/kg by day 14. The amino-DNT compounds were degraded to less than 10 mg/kg by day 84. The diamino-NT compounds (2,4-DANT and 2,6-DANT) increased from 0 mg/kg to 402 mg/kg by day 21. The diamino-NT compounds are subsequently degraded to less than 10 mg/kg by day 84. The Potato Starch treatment highest concentration of azoxy compounds was 14 mg/kg detected on day 7. RDX increased in concentration from 155 mg/kg to 273 mg/kg by day 14 and is subsequently degraded to less than 2 mg/kg by day 35. HMX was reduced from an initial concentration of 81 mg/kg to less than 10 mg/kg by day 49. Overall, total explosives (the sum of all explosive compounds) is degraded from a concentration of 1542 mg/kg to less than 25 mg/kg in 84 days.

Tween 80 and Molasses Treatment (Aerated)

Figure 3-5 is a plot of the average explosive compound concentrations versus time from the duplicate aerated Tween 80 and Molasses reactors. TNT was degraded from an average concentration of approximately 1980 mg/kg to less than 4 mg/kg in 84 days. At day 28, the Potato Starch treatment showed 39 mg/kg TNT (meeting child resident goal). The sum of 4A-DNT and 2A-DNT increased from 119 mg/kg to 640 mg/kg by day 14. The amino-DNT compounds were degraded to less than 11 mg/kg by day 84. The diamino-NT compounds (2,4-NT and 2,6-NT) did not show as significant an increase as their concentration increased from 0 mg/kg to 36 mg/kg on day 28. The Potato Starch treatment showed a significant increase of the azoxy concentration. The Potato Starch treatment azoxy concentration increased from 2 mg/kg to 481 mg/kg by day 21. The azoxy concentration eventually decreased to less than 30 mg/kg by day 84. RDX was degraded from 221 mg/kg to less than 3 mg/kg by day 70. HMX was reduced from 86 mg/kg reduced to less than 4 mg/kg in 84 days. Overall, total explosives (the sum of all explosive compounds) were degraded from a concentration of 2411 mg/kg to less than 48 mg/kg in 84 days.

Sterile Controls (non-aerated)

Figure 3-6 is a plot of the average explosive compound concentrations versus time from the non-aerated Sterile Control biocell reactor. The sterile control reactor showed disappearance of TNT as the concentration decreased from an initial of 1028 mg/kg to a final of 8 mg/kg . The amino-DNT did not show an increase with time which would be indicative of a reductive pathway for TNT. The amino-DNT concentration decreased from 179 mg/kg to 13 mg/kg by day 84. The diamino-NT compounds also did not show a significant increase which indicates that the TNT disappearance was not due to a reductive process. The diamino-NT concentration increased from 0 mg/kg to a maximum of 12 mg/kg. RDX disappeared with time from an initial concentration of 165 mg/kg to less than 10 mg/kg by day 14. HMX was reduced from 111 mg/kg to less than 10 mg/kg by day 21.

Sterile Control (Aerated)

Figures 3-7 are plots of the average explosive compound concentrations versus time from the aerated Sterile Control reactors. As with the case of the non-aerated sterile reactor, this reactor showed disappearance of TNT. The TNT concentration fluctuated from an initial of 452 mg/kg to a final of 1150 mg/kg. The amino-DNT compounds fluctuated from an initial of 166 mg/kg to a final of 45 mg/kg. The amino-DNT did not show an increase which indicates that the dissappearance of TNT was not due to a reductive process. The diamino-NT compounds also did not increase. RDX disappeared with time from an initial concentration of 154 mg/kg to 95 mg/kg. The HMX concentration remained relatively constant with an initial concentration of 76 mg/kg and a final of 70 mg/kg.

Analysis of Results by Explosive Compound

Figure 3-8 is a plot of TNT concentration versus time for all treatments evaluated in the biocell reactors. From Table 5.4.1.2., the Tween 80 and Molasses treatment showed the fastest degradation of TNT with less than 20 mg/kg in 35 days. Both Molasses and Simplot treatments failed to degrade TNT to less than 50 mg/kg in 84 days. The aerated Tween 80 and Molasses resulted in some TNT degradation but it did not achieve results comparable to the Molasses, Simplot, or anaerobic Tween 80 and Molasses. The non-aerated sterile control also showed TNT disappearance resulting in less than 10 mg/kg in 49 days.

Figure 3-9 is a plot of the combined 2A-DNT and 4A-DNT concentration versus time for all treatments evaluated in the bioslurry reactors. All treatments with the exception of both sterile controls and the Tween 80 and Molasses treatments resulted in significant formation of 2A-DNT and 4A-DANT within the first 14 days of the experiment. This data tends to show that TNT is being reduced as the 2A-DNT and 4A-DNT concentration increases as the TNT concentration is decreasing. After formation of the amino compounds, the Tween and Molasses treatment achieved the fastest

degradation to less than 11 mg/kg in 35 days (Table 5.4.1.2.). In comparison, the other treatments reduced 2A-DNT and 4A-DNT to the following levels: Simplot - 7 mg/kg in 84 days, Molasses - 6 mg/kg in 84 days, and Potato Starch - 6 mg/kg in 84 days.

Figure 3-10 is a plot of the combined 2,4-DANT and 2,6-DANT concentration versus time for all treatments evaluated in the bioslurry reactors. All treatments, with the exception of the sterile controls and the aerated Tween 80 and Molasses, showed some formation of 2,4-DANT and 2,6-DANT. The bulk of the diamino compounds were formed during days 14 to 21. The Simplot and Tween 80 and Molasses treatment degraded the 2,4-DANT and 2,6-DANT to less than 20 mg/kg by day 49.

Figure 3-11 is a plot of the combined 4,4-Azoxy and 2,2-Azoxy compounds for all treatments evaluated in the bioslurry reactors. None of the treatments showed significant formation of these complexes.

Figure 3-12. is a plot of RDX concentration versus time for all treatments evaluated in the bioslurry reactors. The Simplot, Potato Starch, and Tween 80 and Molasses treatments showed an increase in RDX concentration during the first 14 days of the experiment. This is possibly due to the shearing of the soil by the mixing action which would tend to make the extraction procedure for analysis more effective. Simplot, Molasses, Potato Starch, and Tween 80 and Molasses treatments degraded RDX to less than 20 mg/kg by day 21. The aerated Tween 80 and Molasses treatment was only able to degrade RDX from an initial concentration of 106 mg/kg to 80 mg/kg.

Figure 3-13. is a plot of HMX concentration versus time for all treatments evaluated in the bioslurry reactors. Eventhough HMX was at the lowest initial concentration of the explosive compounds, it proved to be the most recalcitrant. As with RDX, the HMX concentration increased at day 14. HMX degradation was very slow with the Simplot, Potato Starch, and Molasses treatments achieving less than 10 mg/kg by day 84. The HMX concentration in the Tween 80 and Molasses treatment was degraded to less than 20 mg/kg by day 84.

Bioslurry - Analysis of Results by Treatments

Tween 80 and Molasses Treatment (Aerated)

Figure 3-14 is a plot of the average explosive compound concentrations versus time from the duplicate Tween 80 and Molasses reactors. TNT is degraded from an average concentration of approximately 1350 mg/kg to less than 5 mg/kg in 7 days. The addition of the surfactant Tween 80 greatly enhances the desorption of TNT which is then readily bioavailable for reduction by the microbial population. The sum of 4A-DNT and 2A-DNT increase from 134 mg/kg to 498 mg/kg by day 14. The rapid increase in concentration of the amino compounds shows that TNT is being reduced. The amino-DNT compounds are degraded to less than 10 mg/kg by day 70. The diamino-NT compounds (2,4-DANT and 2,6-DANT) increase from 0 mg/kg to 55 mg/kg by day 28.

The diamino-NT compounds are subsequently degraded to less than 3 mg/kg by day 70. The Tween 80 and Molasses treatment shows no significant formation of the azoxy compounds with less than 4 mg/kg detected on day 21. RDX is degraded from 260 mg/kg to less than 2 mg/kg by day 49. HMX proves to be the most recalcitrant compound with an initial concentration of 98 mg/kg reduced to less than 5 mg/kg in 84 days. Overall, total explosives (the sum of all explosive compounds) is degraded from a concentration of 1854 mg/kg to less than 15 mg/kg in 84 days.

No Additives Treatment (Aerated)

Figure 3-15 is a plot of the average explosive compound concentrations versus time from the duplicate No Additive reactors. TNT remains at a relatively constant concentration throughout 70 days of the experiment. The TNT concentration on day 84 shows a decrease but is most likely due to the heterogeniety of the soil. The amino-DNT compounds show a slight increase on days 7 and 14 but this is most likely due to the mixing action of the reactor enabling the extraction process for analysis to be more effective. HMX and RDX concentrations also show a slight increase during the first two weeks and then the concentrations remain relatively constant throughout the experiment. The No Additives treatment does not appear to stimulate biological activity that degrades explosive compounds. The fact that the No Additives treatment contain nitrogen and phosphorus but no carbon indicates that a carbon source (cometabolite) is necessary for explosive degradation.

Simplot Treatment (Anaerobic)

Figure 3-16 is a plot of the average explosive compound concentrations versus time from the duplicate Simplot reactors. TNT is degraded from an average concentration of approximately 1590 mg/kg to less than 9 mg/kg in 84 days. However, the Simplot treatment showed less than 30 mg/kg TNT concentration at 49 days which is less than the treatment goal for a child of 39 mg/kg. The sum of 4A-DNT and 2A-DNT increased from 143 mg/kg to 574 mg/kg by day 14. The rapid increase in concentration of the amino compounds shows that TNT is being reduced. The amino-DNT compounds were degraded to less than 70 mg/kg by day 84. The diamino-NT compounds (2,4-DANT and 2,6-DANT) did not show a significant increase during the sampling events. The Simplot treatment showed a significant formation of the azoxy compounds with the concentration increasing from 1 mg/kg to 389 mg/kg by day 21. The azoxy concentration eventually decreased to 101 mg/kg by day 84. RDX is degraded from 262 mg/kg to less than 44 mg/kg by day 84. HMX proved to be the most recalcitrant compound with an initial concentration of 92 mg/kg reduced to less than 62 mg/kg in 84 days. Overall, total explosives (the sum of all explosive compounds) were degraded from a concentration of 2092 mg/kg to less than 305 mg/kg in 84 days.

Simplot Treatment with 4 hours mixing daily (Anaerobic)

Figure 3-17 is a plot of the average explosive compound concentrations versus time from the duplicate Simplot with 4 hr mix reactors. TNT is degraded from an average concentration of approximately 2240 mg/kg to less than 4 mg/kg in 70 days. At day 49, the Simplot with 4 hr mix treatment showed less than 49 mg/kg TNT. No sample was taken at days 56 and 63 so it is likely that these reactors met the 39 mg/kg treatment goal for a child during this timeframe. The sum of 4A-DNT and 2A-DNT increased from 106 mg/kg to 655 mg/kg by day 28. The amino-DNT compounds were degraded to less than 7 mg/kg by day 84. The diamino-NT compounds (2,4-DANT and 2,6-DANT) did not show as significant an increase as their concentration increased from 0 mg/kg to 61 mg/kg on day 49. The Simplot with 4 hr mix treatment did not show as significant an increase of the azoxy concentration as the Simplot treatment. The Simplot with 4 hr mix treatment azoxy concentration increased from 1 mg/kg to 166 mg/kg by day 21. The azoxy concentration eventually decreased to less than 8 mg/kg by day 70. RDX was degraded from 212 mg/kg to less than 7 mg/kg by day 49. HMX again proved to be the most recalcitrant compound with an initial concentration of 78 mg/kg reduced to less than 3 mg/kg in 70 days. Overall, total explosives (the sum of all explosive compounds) were degraded from a concentration of 2641 mg/kg to less than 18 mg/kg in 84 days.

Potato Starch Treatment (Anaerobic)

Figure 3-18 is a plot of the average explosive compound concentrations versus time from the duplicate Potato Starch reactors. TNT was degraded from an average concentration of approximately 1980 mg/kg to less than 4 mg/kg in 84 days. At day 28, the Potato Starch treatment showed 39 mg/kg TNT (meeting child resident goal). The sum of 4A-DNT and 2A-DNT increased from 119 mg/kg to 640 mg/kg by day 14. The amino-DNT compounds were degraded to less than 11 mg/kg by day 84. The diamino-NT compounds (2,4-NT and 2,6-NT) did not show as significant an increase as their concentration increased from 0 mg/kg to 36 mg/kg on day 28. The Potato Starch treatment showed a significant increase of the azoxy concentration. The Potato Starch treatment azoxy concentration increased from 2 mg/kg to 481 mg/kg by day 21. The azoxy concentration eventually decreased to less than 30 mg/kg by day 84. RDX was degraded from 221 mg/kg to less than 3 mg/kg by day 70. HMX was reduced from 86 mg/kg reduced to less than 4 mg/kg in 84 days. Overall, total explosives (the sum of all explosive compounds) were degraded from a concentration of 2411 mg/kg to less than 48 mg/kg in 84 days.

Sterile Controls (non-aerated)

Figures 3-19 are plots of the average explosive compound concentrations versus time from the non-aerated Sterile Control reactors. The initial concentrations of the explosive compounds are the average of the 4 samples taken from the Simplot, Potato Starch, No Additives, and Tween and Molasses reactors. The sterile control reactor showed disappearance of TNT; however, the TNT concentration fluctuated with time

which is probably due to sample heterogeniety. The TNT concentration fluctuated from an initial of 1800 mg/kg to a final of 620 mg/kg. The amino-DNT compounds fluctuated from an initial of 125 mg/kg to a low of 38 mg/kg with a final of 67 mg/kg. The amino-DNT did not show an increase which indicates that the dissappearance of TNT was due to a reductive process. The diamino-NT compounds never showed a significant increase either which is further proof that the TNT disappearance was not due to reductive processes. The diamino-NT concentration increased from 0 mg/kg to 18 mg/kg and then reduced to 0 mg/kg at day 84. RDX disappeared with time from an initial concentration of 238 mg/kg to 45 mg/kg. HMX was reduced by half from an initial concentration of 89 mg/kg to 45 mg/kg.

Sterile Controls (Aerated)

Figures 3-20 are plots of the average explosive compound concentrations versus time from the non-aerated Sterile Control reactors. The initial concentrations of the explosive compounds are the average of the 4 samples taken from the Simplot, Potato Starch, No Additives, and Tween and Molasses reactors. As with the case of the non-aerated sterile reactor, this reactor showed disappearance of TNT. The TNT concentration fluctuated from an initial of 1800 mg/kg to a final of 418 mg/kg. The amino-DNT compounds fluctuated from an initial of 125 mg/kg to a low of 56 mg/kg with a final of 60 mg/kg. The amino-DNT did not show an increase which indicates that the dissappearance of TNT was due to a reductive process. The diamino-NT compounds also did not show a significant increase. The diamino-NT concentration increased from 0 mg/kg to 16 mg/kg and then reduced to less than 1 mg/kg at day 70. RDX disappeared with time from an initial concentration of 238 mg/kg to 40 mg/kg. HMX was reduced by half from an initial concentration of 89 mg/kg to 42 mg/kg.

Analysis of Results by Explosive Compound

Figure 3-21 is a plot of TNT concentration versus time for all treatments evaluated in the bioslurry reactors. One of the two most interesting results is that the TNT concentration in the No Additives treatment remained relatively constant throughout the experiment. In effect, the No Additives treatment served as a control for the experiment. The other interesting result is that the Tween 80 and Molasses treatment resulted in almost complete degradation of TNT. The time required for each treatment to degrade TNT to the resident child treatment goal of 39 mg/kg is as follows: Tween and Molasses - 7 days, Simplot - 49 days, Simplot with 4 hr mix - 70 days, Potato Starch - 28 days, and No Additives never reached the goal.

Figure 3-22 is a plot of the combined 2A-DNT and 4A-DNT concentration versus time for all treatments evaluated in the bioslurry reactors. All treatments with the exception of the sterile controls and no additives show significant formation of 2A-DNT and 4A-DANT within the first 14 days of the experiment. This data tends to show that TNT is being reduced as the 2A-DNT and 4A-DNT concentration increases as the TNT concentration is decreasing. After formation of the amino compounds, the Tween and

Molasses treatment achieved the fastest degradation to less than 20 mg/kg in 49 days. In comparison, the other treatments reduced 2A-DNT and 4A-DNT to the following levels: Simplot - 66 mg/kg in 84 days, Simplot with 4 hr mix - 7 mg/kg in 84 days, and Potato Starch - 10 mg/kg in 84 days. The concentration of 2A-DNT and 4A-DNT remained relatively constant in the No Additives treatment.

Figure 3-23 is a plot of the combined 2,4-DANT and 2,6-DANT concentration versus time for all treatments evaluated in the bioslurry reactors. All treatments showed some formation of 2,4-DANT and 2,6-DANT. The bulk of the diamino compounds were formed during days 7 to 49. Tween and Molasses showed the fastest formation of the diamino compounds, which is expected as it achievied the quickest degradation of TNT. All treatments degraded the 2,4-DANT and 2,6-DANT to less than 10 mg/kg by day 70. This data further supports the fact that TNT is being reduced as the diamino compounds tend to form subsequent to the amino compounds.

Figure 3-24 is a plot of the combined 4,4-Azoxy and 2,2-Azoxy compounds for all treatments evaluated in the bioslurry reactors. Only the variations of the Simplot process showed the formation of these complexes. The greatest formation occured during days 14 to 28 with Potato Starch having the highest concentration at 481 mg/kg. The Simplot with 4 hr mix showed the lowest formation of azoxy compounds of the three Simplot treatment variations. The Simplot with 4 hr mix showed a maximum concentration of 166 mg/kg which was completely degraded by day 49. Simplot and the Potato Starch treatments reduced the azoxy concentration to 100 mg/kg or less by day 84. It is interesting to note that the formation of azoxy compounds has been postulated to occur under aerobic conditions. It is possible that due to the mixing action of the bioslurry air diffused into the slurry. However, the Tween and Molasses treatment was aerated and did not show the formation of the azoxy compounds.

Figure 3-25 is a plot of RDX concentration versus time for all treatments evaluated in the bioslurry reactors. The Simplot, Potato Starch, No Additives, and Simplot with 4 hr mix treatments showed an increase in RDX concentration during the first 14 days of the experiment. This is possibly due to the shearing of the soil by the mixing action which would tend to make the extraction procedure for analysis more effective. Both Tween and Molasses and Simplot with 4 hr mix treatments degraded RDX to less than 10 mg/kg by day 49. Potato Starch degraded RDX to less than 10 mg/kg by day 70 and the Simplot process degraded RDX to 44 mg/kg by day 84. The resident child goal for RDX is 230 mg/kg and all treatments achieved that concentration by day 21.

Figure 3-26 is a plot of HMX concentration versus time for all treatments evaluated in the bioslurry reactors. Eventhough HMX was at the lowest initial concentration of the explosive compounds, it proved to be the most recalcitrant. As with RDX, the HMX concentration increased at day 7. HMX degradation was very slow with the Simplot with 4 hr mix, Potato Starch, and Tween and Molasses treatments achieving less than 5 mg/kg by day 84. The HMX concentration in the No Additives treatment remained relatively constant throughout the experiment. The resident child goal for HMX

is 3900 mg/kg which is far greater than any initial sample taken. Thus, HMX degradation should not critical to meeting site clean-up goals.

Analysis of Explosives by Oxidation-Reduction Potential

Figures 3-27 through 3-39 are combination plots of oxidation-reduction potential (ORP), oxygen uptake rate (OUR), dissolved oxygen (DO), and explosives concentration. Anaerobic reactors were not measured for DO and OURs were not determined. These plots have been stacked in order to determine whether explosives degradation is dependent on redox potential. Generally, redox potentials of less than -200 mv are considered anaerobic environments, + 200 mv is aerobic and a range between the two (-100 to 0 mv) is considered anoxic (oxygen containing species present such as nitrate (NO_3) but no dissolved oxygen).

The ORP, DO, and OUR indicate that the aerated No Additives bioslurry reactors (Figure 3-27) maintained aerobic conditions. All explosives concentrations remain relatively constant throughout the course of the experiment with a slight decrease in TNT concentration at day 84 which is probably due to soil heterogeniety. It is apparent that none of the compounds are being degraded under aerobic conditions as no carbon source was provided to induce cometabolic degradation.

In contrast, the anaerobic Molasses biocell (Figure 3-28) shows a steady decrease in ORP with time. The Molasses serves as a cometabolic carbon source that microbes use for growth and energy. As the molasses is used as a substrate, the explosives compounds are degraded. Initially, aerobic microbes oxidize molasses and use dissolved oxygen as the terminal electron acceptor (converting DO to CO_2). This 'consumes' the oxygen in the water and serves as the driving force to anaerobic conditions.

Figures 3-29 through 3-31 are all versions of the Simplot process. The degradation of explosive compounds in the Simplot biocell (figure 3-29) is very similiar to the Molasses biocell (figure 3-28). Of note is length of time required to totally degrade TNT under anaerobic conditions in both processes. The Simplot bioslurry (figure 3-30) was anoxic and did not reach anaerobic conditions until the end of the experiment Of note under anoxic conditions is the fact that TNT is reduced faster than under anaerobic conditions yet the transformation products then 'linger' until anaerobic conditions are achieved. This trend is supported by the results of the Simplot with 4 hr mix (figure 3-31) as the reactor is initially anoxic and then proceeds to anaerobic conditions. TNT is quickly reduced under anoxic conditions, and RDX, HMX, and the TNT transformation products are degraded under anaerobic conditions.

The results of the Potato Starch treatment are similiar to the Simplot treatments in that TNT was quickly reduced under anoxic conditions in the bioslurry (figure 3-32) and the transformation products are degraded under anaerobic conditions in the biocell (figure 3-33).

The Simplot processes showed highest concentration of A-DNT and DA-NT compounds which may be indicative of a process that more clearly results in the stepwise reduction of TNT to amino and diamino intermediate compounds. Of note, is that the azoxy compound was only found in the versions of the Simplot process (Simplot and Potato Starch) under anoxic conditions. It appears to form under anoxic conditions and is degraded under anaerobic conditions. In those reactors that proceeded to anaerobic conditons the azoxy compound was not identified.

As illustrated by the figures reviewed thus far, the bioslurry reactors did not reach anaerobic conditions as quickly as the biocell reactors. The difference in redox potential between the biocells and bioslurries is a function of oxygen transfer. The biocells were occasionally mixed (a few times weekly) whereas the bioslurries were continuously mixed 24 hours a day. The mixing increased the rate of oxygen transfer and it was high enough to exceed the respiration rate (oxygen consumption) of the microorganisms thus leading to anoxic conditions. The biocells did not have continuous mixing and the microbial respiration rate exceeded the oxygen transfer rate which drove the reactor to the anaerobic state.

The 'landfarm in a can' treatment (aerated Tween 80 and Molasses biocell - figure 3-34) illustrates the inability of the native consortia to degrade TNT and other explosive compounds in this reactor. The fluctuations in explosive concentrations are probably due to soil heterogeniety rather than biodegradation as the initial increase in transformation products does not 'linger' over the course of the experiment.

The anaerobic Tween 80 and Molasses biocell (figure 3-35) shows the same relationship between redox potential and explosives concentration as seen previously in anaerobic reactors. The TNT is slowly reduced and the transformation products, RDX, and HMX, are quickly reduced under anaerobic conditions.

Of note, is the fact that the aerated Tween 80 and Molasses bioslurry (figure 3-36) tended to anaerobic conditions and was anoxic throughout the majority of the experiment. The Tween 80 enhanced the desorption of TNT from soil allowing it to be rapidly reduced within 7 days. All of the transformation products, RDX, and HMX, are degraded as anerobic conditions are reached.

TNT reduction does not appear to be a function of ORP as it disappears in all cometabolic treatments. TNT transformation products and RDX appear to be correlated to ORP as those compounds 'linger' for extended periods under anoxic conditions and disappear under anaerobic conditions (Simplot bioslurry, Potato Starch bioslurry, Potato Starch biocell, Tween 80 and Molasses bioslurry, Molasses biocell).

Figures 3-37 through 3-39 are the sterile controls for biocell and bioslurry reactors. Figures 3-38 and 3-39 show TNT fluctuating with time yet no significant formation of transformation products. Figure 3-37 shows a first-order decay of TNT and all other explosive compounds.

In order to better understand the phenomena occuring in the reactor that showed dissappearance of explosive compounds side studies were conducted to examine TNT and mercuric chloride interactions. TNT at a concentration of 11.3 ppm was mixed with 0.15% (wt/wt) mercuric chloride in DDI water. There was no decrease in TNT concentration after 10 days. The temperature was elevated to 80 C for 12 hours and there was also no decrease in TNT concentration. Two hundred grams of montmorillonite clay was added to TNT in DDI water and the TNT concentration was reduced by one-half in a day (losses due to sorption as no transformation products were formed). The montmorillonite clay was then added to a solution of TNT and mercuric chloride and the TNT concentration was reduced from 11.3 ppm to 0.10 ppm in one day (losses in this case due to sorption and degradation as some transformation products were formed). Thus, in a contiuous mixed system as a bioslurry (figures 3-25 and 3-26) it is expected that the TNT concentration would vary as the compound is sorbing and desorbing from the soil matrix as a result of the interaction between mercuric chloride, clay, and TNT. The first order decay shown in the biocell (figure 3-24) is most likely due to sorption. The cell is not continuously mixed which would enhance the sorption/desorption process seen in the biocell.

Kinetic Analysis

The data provided on the plots presented thus far is important to obtain a qualitative feel for the relative explosive degradative ability of the different treatments, reactors, and electron acceptor conditions. However, in order to quantitatively analyze the data, the degradation kinetics must be determined in order to define certain variables such as the rate coefficient, half-life, and soil retention time. An analysis of the reaction kinetics allows this information to be determined.

Typically, first order kinetics are assumed when analyzing degradation of organic compounds within biological systems. In order to verify this assumption, all explosives compound data was plotted against zero, first, second and n^{th} order kinetic plots to determine the actual degradation kinetics for each treatment and explosive compound. The data was regressed in order to determine the best fit.

The reaction orders for all of the treatments varied between 0.6 to 1.1. Based on these results, first order degradation was assumed for all cometabolic processes. The first order expression was then used to solve for the rate coefficient (k), half-life ($t_{1/2}$), and soil retention time (SRT) based on an average for each treatment. The resulting data is provided in the tables below.

Within the biocell reactors (table x), the surfactant amended process (Tween 80 and Molasses) achieved a TNT degradation rate twice that of the other processes examined. The Molasses, Simplot, Potato Starch (Simplot without propietary consortia) all performed comparably. The Tween 80 and Molasses aerated biocell was the least effective due to very limited contaminant bioavailability. This reactor configuration is very similiar to 'landfarming in a can' and thus did not have water present to enhance the bioavailability of the explosive compounds. Additionally, due to the clay content, the soils formed small balls which encapsulates explosive compounds in the interior.

Table 3-1. Rate coefficient, correlation coefficient, half-life, and soil retention time for explosive compounds in biocell reactors utilizing various ammendments and electron-acceptor conditions.

Contaminant	Treatment	Rate coefficient k ($days^{-1}$)	Correlation coefficient r^2	Half-life $t_{1/2}$ (days)	Soil Retention Time (SRT, days)
TNT	Tween 80 & Molasses (anaerobic)	0.17	0.86	4.2	23.5
	Molasses (anaerobic)	0.07	0.60	10.1	57
	Simplot (anaerobic)	0.05	0.70	12.7	71.5
	Potato Starch (anaerobic)	0.07	0.92	10.4	58.9
	Tween 80 & Molasses (aerated)	0.023	0.47	30.1	230

The surfactant-amended treatment also performed best in the bioslurry reactors. The rate coefficient for the Tween 80 and Molasses treatment was approximately ten times larger than other processes which indicates a degradation rate that is ten times faster than other processes. All versions of Simplot (Simplot, Potato Starch, and Simplot with 4 hr mix) had nearly identical rate coefficients and thus, treatment time. Based on the comparable performance, it appears that the addition of the propietary Simplot organisms do not provide much benefit in the remediation of WPNSTA Yorktown soils. The No Additives treatment did not degrade TNT as no carbon was added to induce the cometabolic process. The first order rate coefficient was determined just to illustrate its inability to remediate TNT contaminated soil.

Table 3-2. Rate coefficient, correlation coefficient, half-life, and soil retention time for explosive compounds in bioslurry reactors utilizing various ammendments and electron-acceptor conditions.

Contaminant	Treatment	Rate coefficient k ($days^{-1}$)	Correlation coefficient r^2	Half-life $t_{1/2}$ (days)	Soil Retention Time (SRT, days)
TNT	Simplot (anaerobic)	0.071	0.98	9.7	54.8
	Simplot with 4 hr mix daily (anaerobic)	0.079	0.88	8.7	49
	Potato Starch (anaerobic)	0.079	0.94	8.7	49
	Tween 80 & Molasses (aerated)	0.56	0.94	1.2	7.8
	No Additives (aerated)	0.0018	0.25	385	2174

Table 3-3 is a comparison of first order rate coefficients for all treatments and the major contaminants found in WPNSTA Yorktown soil (TNT, RDX, and HMX). As previously discussed, the surfactant amended reactors degrade TNT the quickest. The rate coefficients are similiar for all versions of Simplot irregardless of reactor configuration (i.e. biocell or bioslurry) which indicates that the benefit of continuous mixing in these processes is negligible. The Tween 80 and Molasses, Simplot, and Potato Starch treatments had the highest rate coefficients for RDX degradation in biocells. Due to the intermittant mixing that occured in the biocell reactors, RDX degradation may be a function of redox potential as the biocells were generally had lower redox potentials than the bioslurry reactors. The degradation rate coefficient for HMX was lower than the other rate coefficients and is indicative of its recalcitrance to degradation.

Table 3-3. Comparison of first order reaction coefficients for the degradation of explosive compounds within bioslurry and biocell reactors utilizing various ammendments under different electron acceptor conditions.

		TNT		RDX		HMX	
Process	Reactor	k ($days^{-1}$)	r^2	k ($days^{-1}$)	r^2	k ($days^{-1}$)	r^2
Tw 80 & Molasses	Biocell (anaerobic)	0.17	0.86	0.16	0.83	0.019	0.72
Tw 80 & Molasses	Biocell (aerated)	0.023	0.47	0.008	0.56	0.004	0.87
Tw 80 & Molasses	Bioslurry	0.56	0.94	0.1	0.9	0.015	0.68
Simplot	Biocell	0.05	0.7	0.14	0.68	0.019	0.72
Simplot	Bioslurry	0.07	0.98	0.009	0.55	0.005	0.19
Simplot w/ 4hr mix	Bioslurry	0.08	0.89	0.08	0.86	0.05	0.72
Potato Starch	Biocell	0.07	0.92	0.18	0.91	0.06	0.83
Potato Starch	Bioslurry	0.08	0.95	0.06	0.66	0.009	0.62
Molasses	Biocell	0.07	0.6	0.09	0.88	0.06	0.93
No Additives	Bioslurry	0.002	0.25	0.01	0.66	0.005	0.49

CONCLUSIONS

Explosives contaminated soil from WPNSTA can be successfully treated using biological systems under aerobic, anoxic, or anaerobic conditions.

All cometabolic treatments resulted in the reduction of TNT. TNT degradation followed the stepwise reduction of the nitro groups through the amino compounds to the diamino compounds. The reduction of TNT was faster under aerobic conditions as compared to anoxic/anaerobic conditions.

The degradation of TNT transformation products (A-DNT and DA-NT), RDX, and HMX, was a function of redox potential as their degradation was much faster under anoxic/anaerobic conditions as compared to aerobic conditions.

The surfactant amended Tween 80 and Molasses reactors resulted in the fastest reduction of TNT in both bioslurry and biocell reactors. The rate coefficient was approximately ten times larger than any other treatment examined and thus, the treatment was approximately 10 times faster than any other. It appears that the surfactant overcomes mass transfer limitations as the TNT is rapidly reduced within 7 days.

The other cometabolic treatments examined were successful in degrading TNT. Their results were essentially the same. All versions of the Simplot process achieved comparable results irregardless of both the mixing energy provided (biocell vs. bioslurry vs. 4 hr mix) and whether the exogenous organisms were added (Simplot vs. Potato Starch). All of these treatments had equivalent TNT degradation rate coefficients with the only exception being the Tween 80 & Molasses (biocell-aerated) and No Additives treatment (aerated-bioslurry). The Tween 80 and Molasses aerated biocell was ineffective due to the limited bioavailability of the explosive compounds in this reactor. The No Additives aerated bioslurry was not capable of degrading TNT due to the fact that no carbon source was added for the cometabolic process.

It appears that a coupled aerobic-anoxic/anaerobic operating regime may result in the optimum treatment condition.This is due to the fact that TNT was most rapidly reduced under aerobic conditions and its transformation products, RDX, and HMX were most rapidly reduced under anoxic/anaerobic conditions.

RECOMMENDATIONS

The surfactant amended process should be considered for pilot scale use as it resulted in the reduction of TNT ten times faster than any other process. All other cometabolic processes degraded TNT at approximately the same rate which is indicative of a mass transfer limited reaction. The surfactant overcomes the mass transfer limitation by both reducing the surface tension and forming micelles. Reducing the surface tension increases the mass transfer of the contaminant. The micelle is a pseudophase into which the TNT can partition thus increasing the concentration above solubility limits.

Further research should examine the combination of aerobic/anoxic processes. The surfactant-amended aerobic process resulted in the quickest reduction of TNT yet the transformation products, RDX, and HMX were degraded faster under anoxic/anaerobic conditions. It is envisioned that an initial aerobic step followed by the onset of anoxic and/or anaerobic conditions will result in the fastest degradation of all explosive compounds in the WPNSTA Yorktown soil.

NOTE: Figures 1-1 through 1-6 (pages 41 - 46) are not included in this draft version.

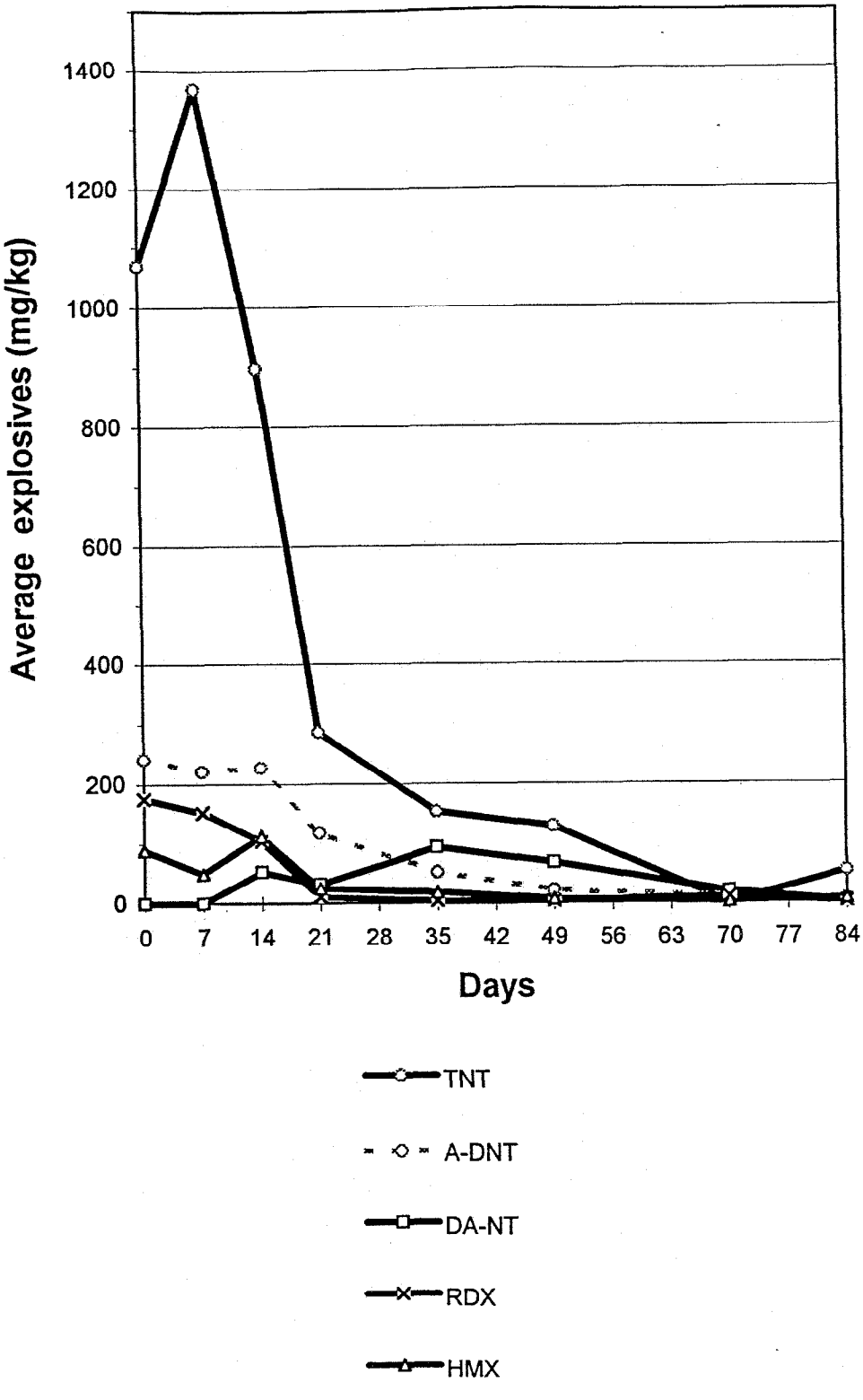


Figure 3-1. Degradation of explosive compounds in anaerobic Molasses biocell reactors.

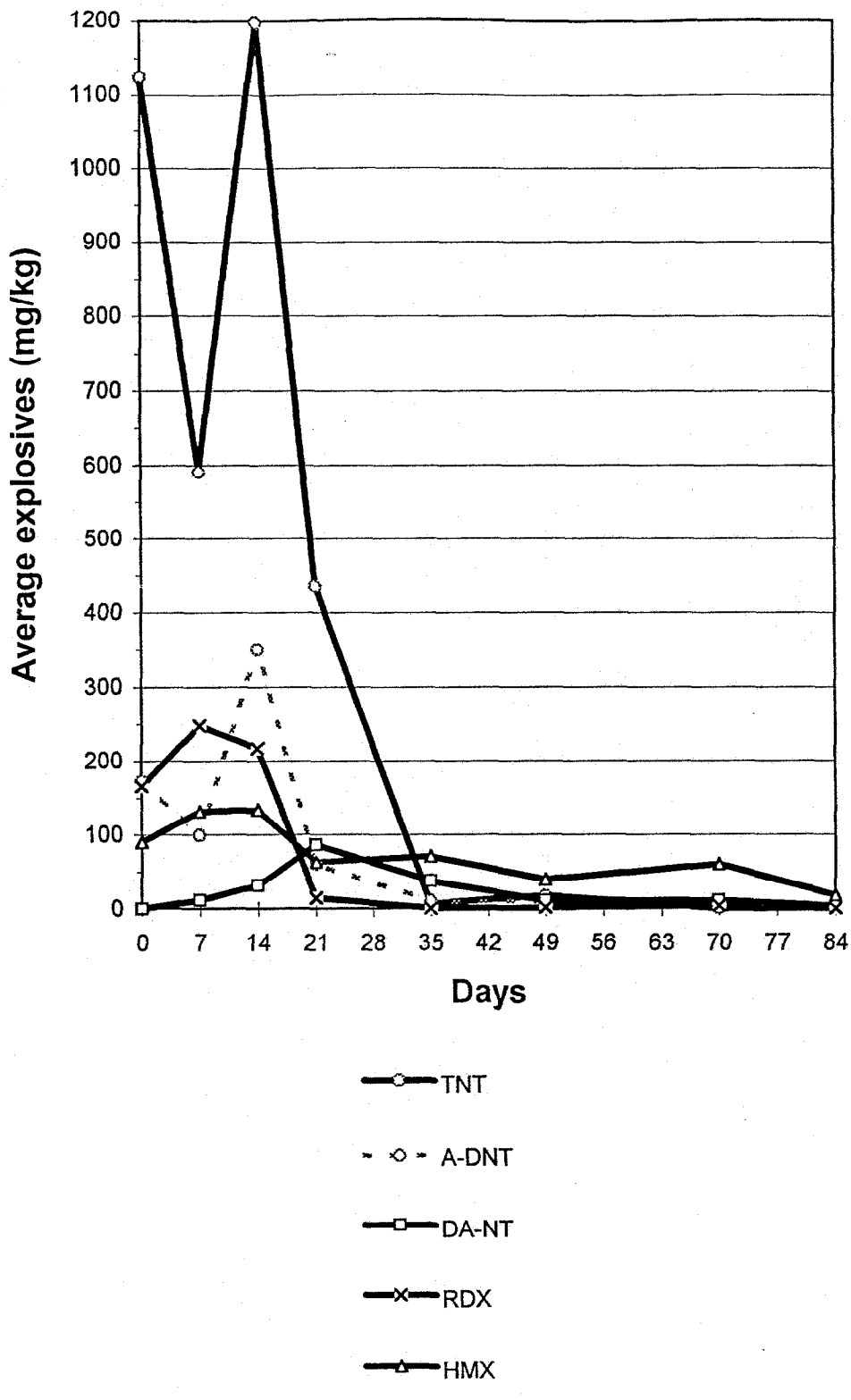


Figure 3-2. Degradation of explosive compounds in anaerobic Tween 80 and Molasses biocell reactors

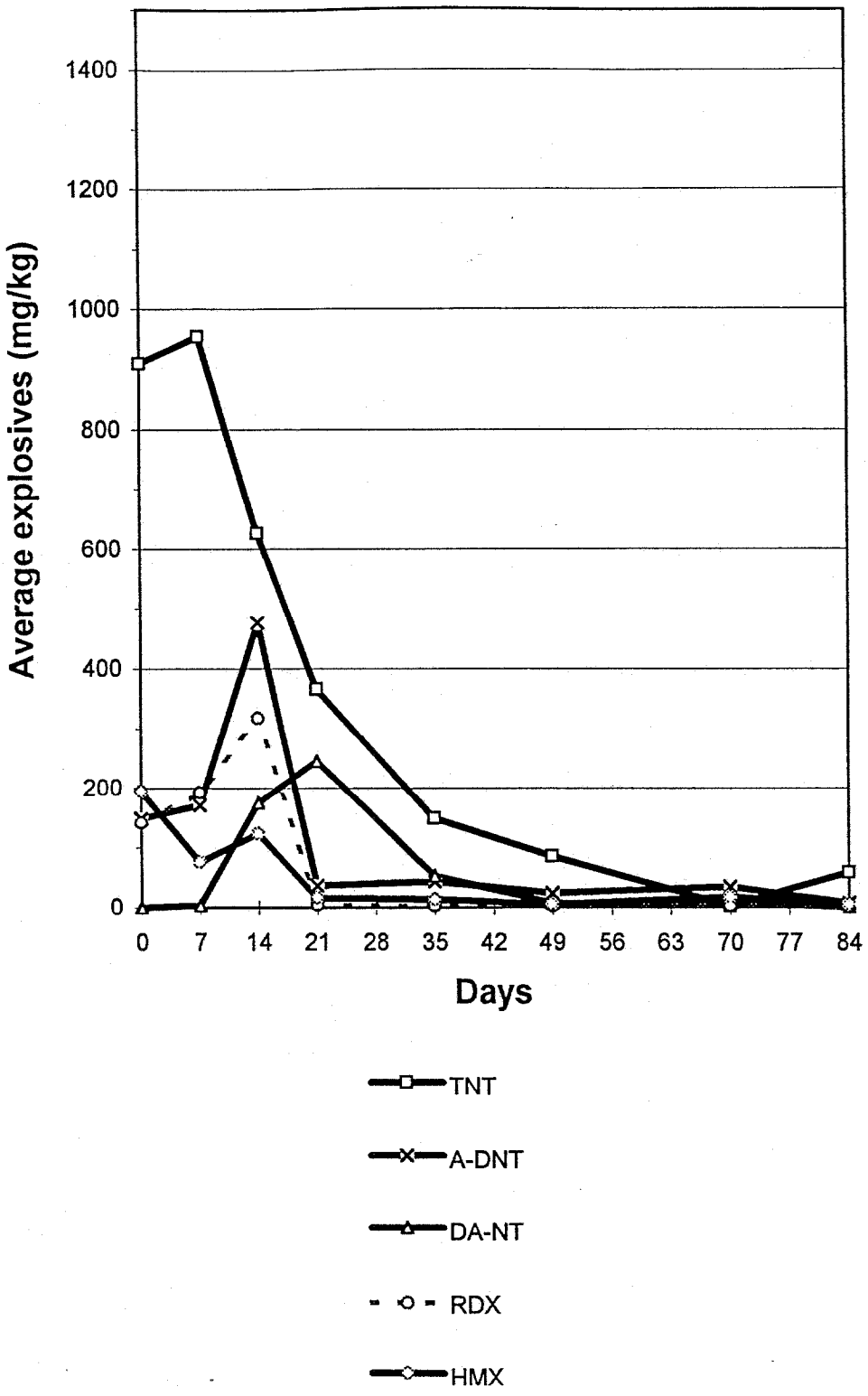


Figure 3-3. Degradation of explosive compounds in anaerobic Simplot biocell reactors

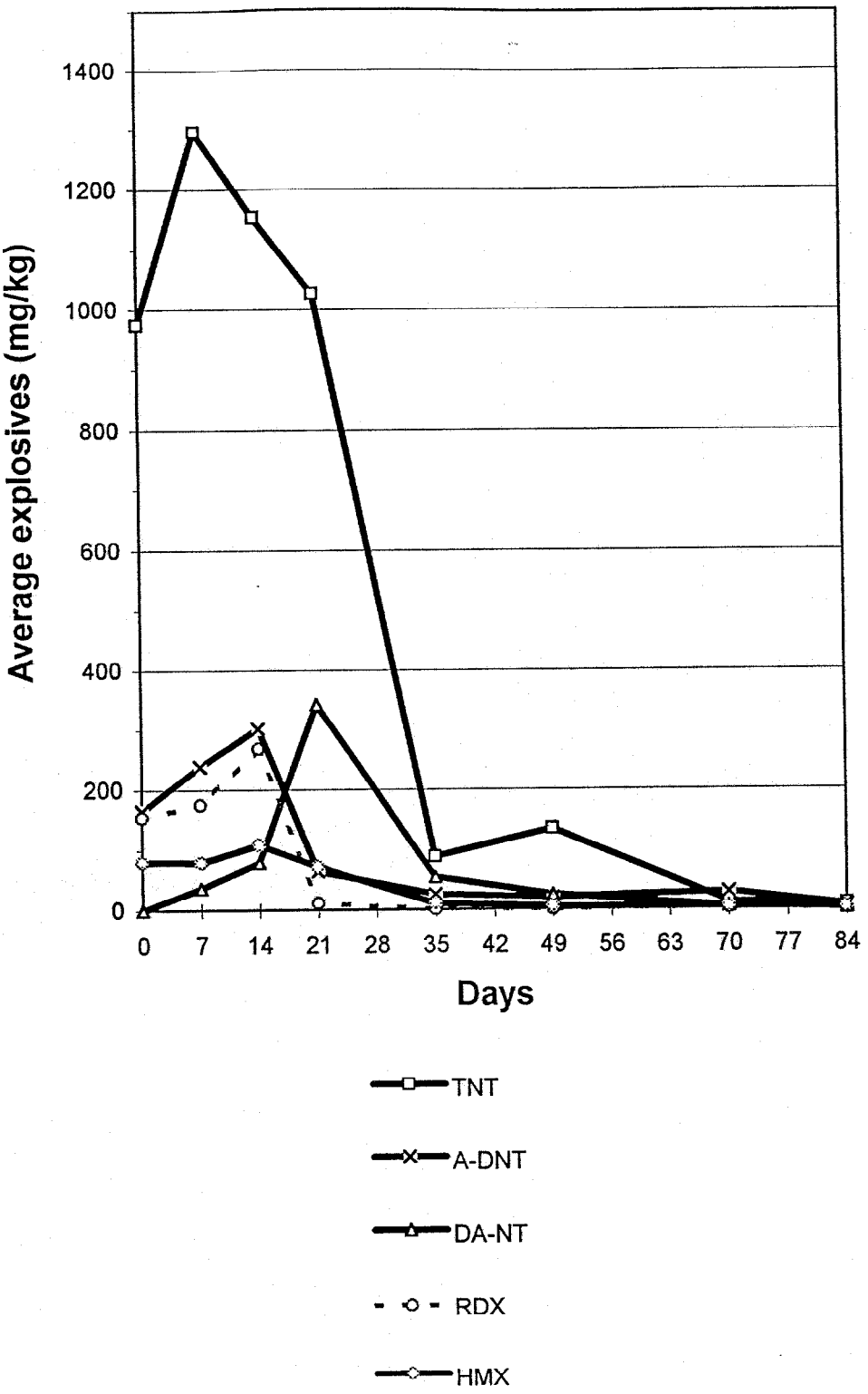


Figure 3-4. Degradation of explosive compounds in anaerobic Potato Starch biocell reactors

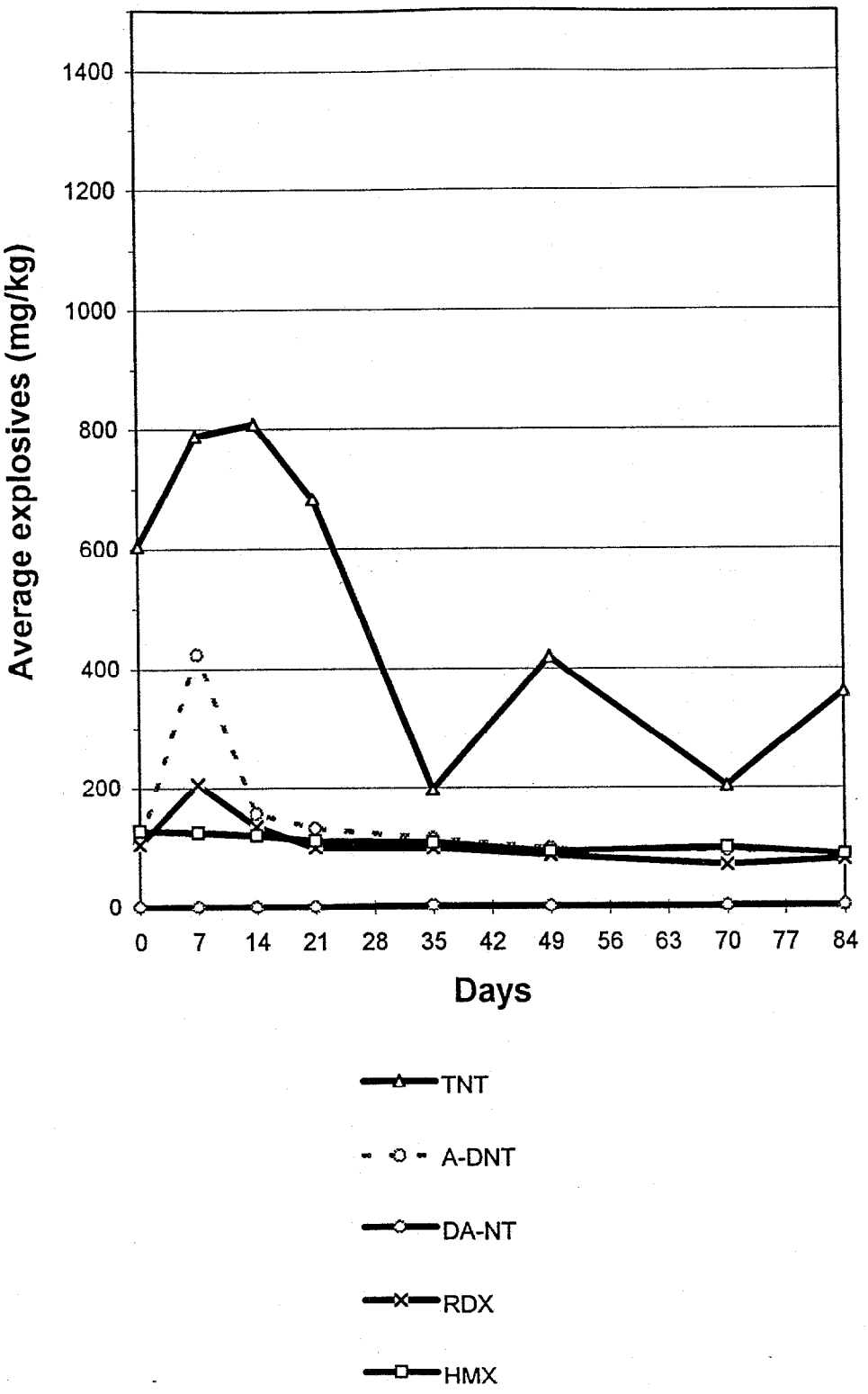


Figure 3-5. Degradation of explosive compounds in aeratedTween 80 and Molasses biocell reactors

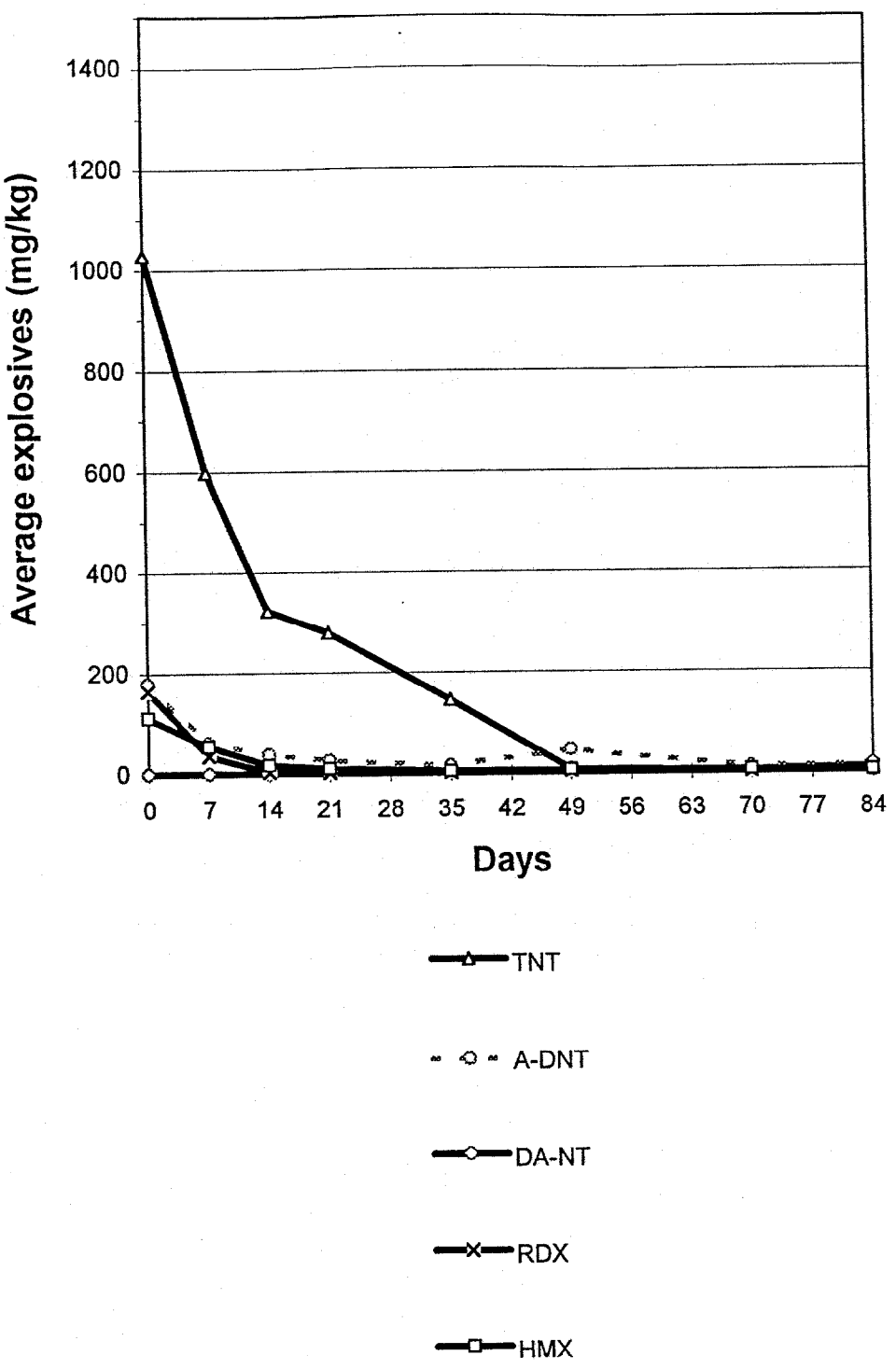


Figure 3-6. Concentration of explosive compounds in non-aerated Sterile Control biocell reactors

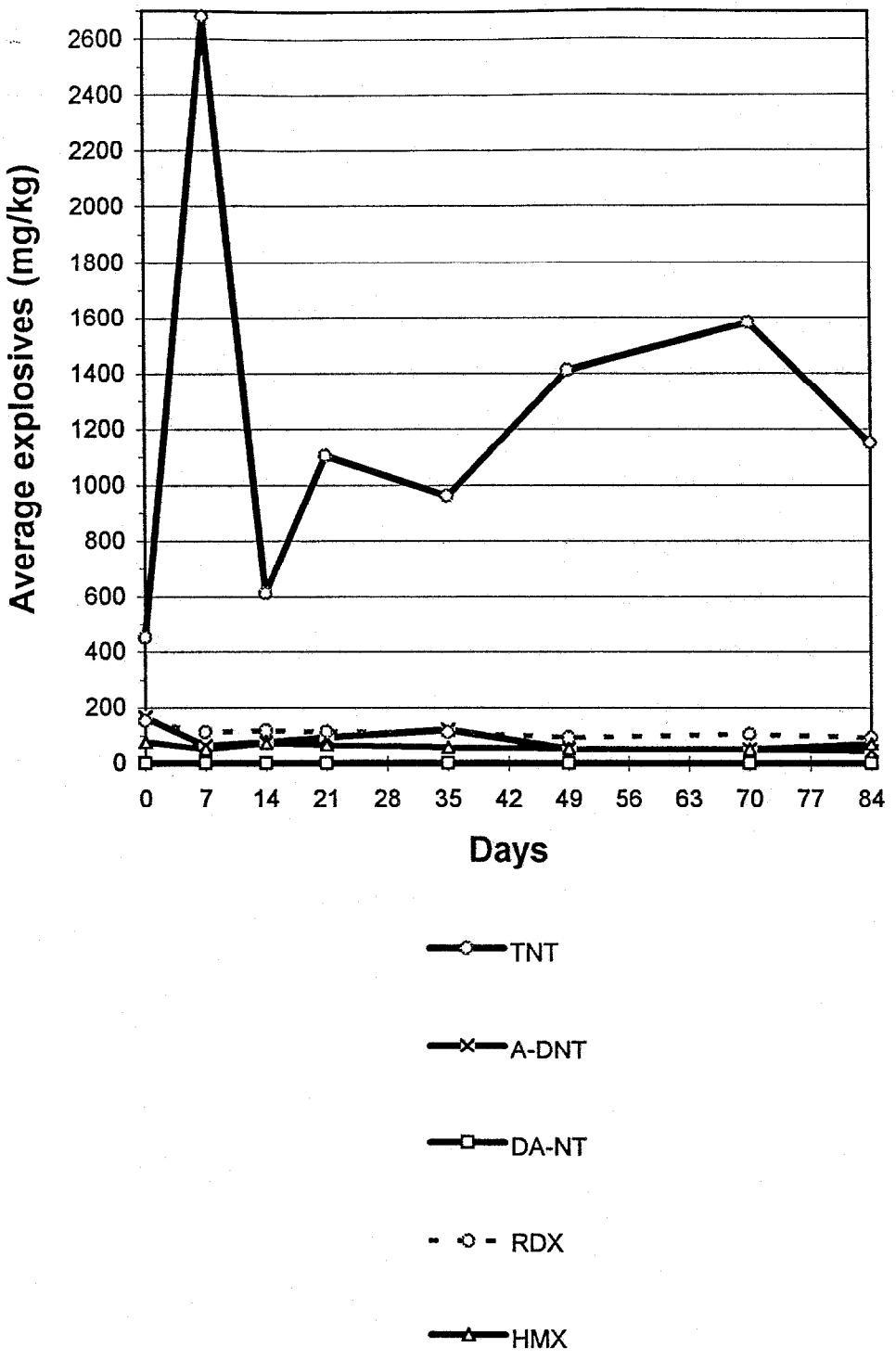


Figure 3-7. Concentration of explosive compounds in aerated Sterile Control biocell reactors

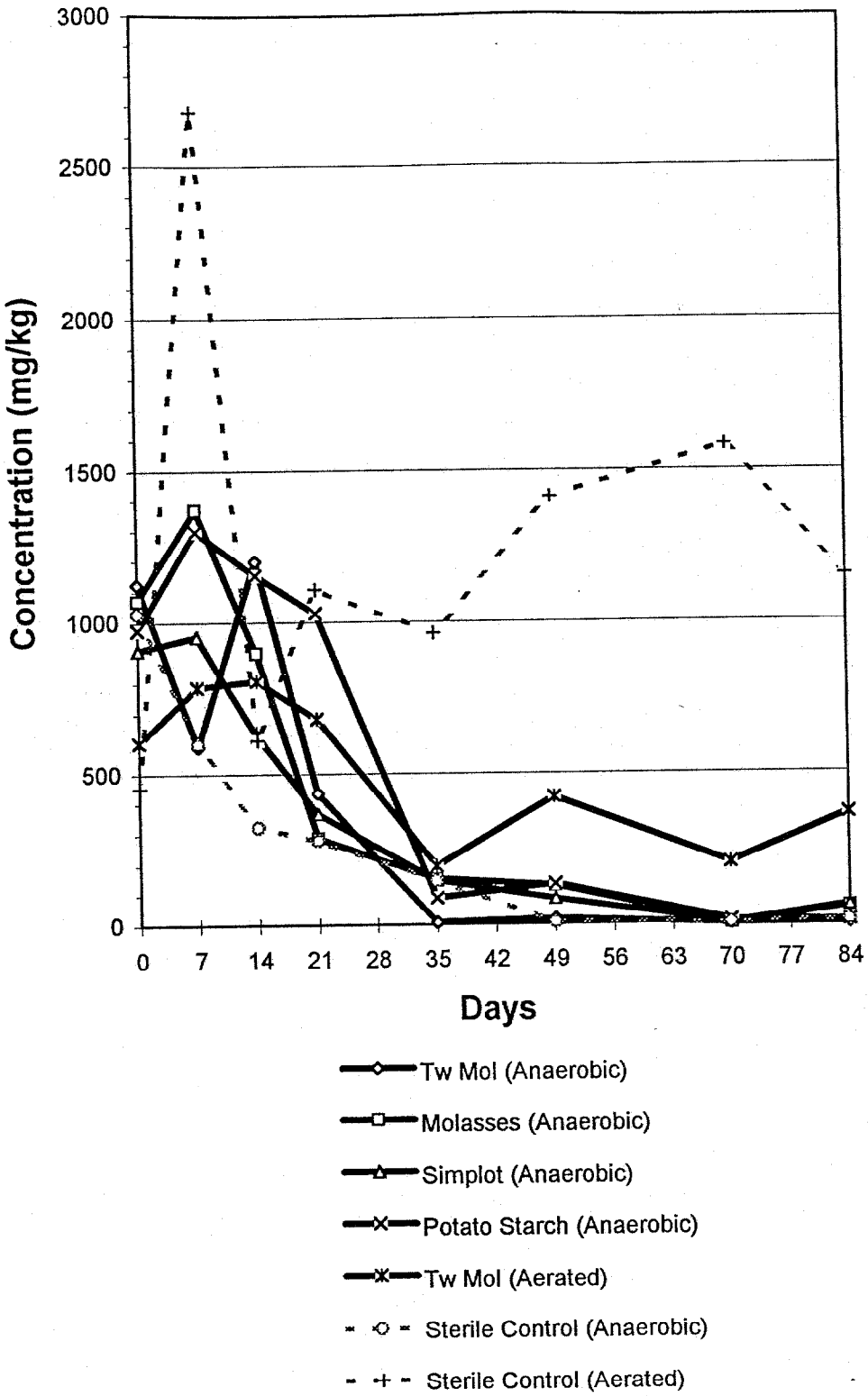


Figure 3-8. Degradation of TNT in anaerobic and aerated biocell reactors

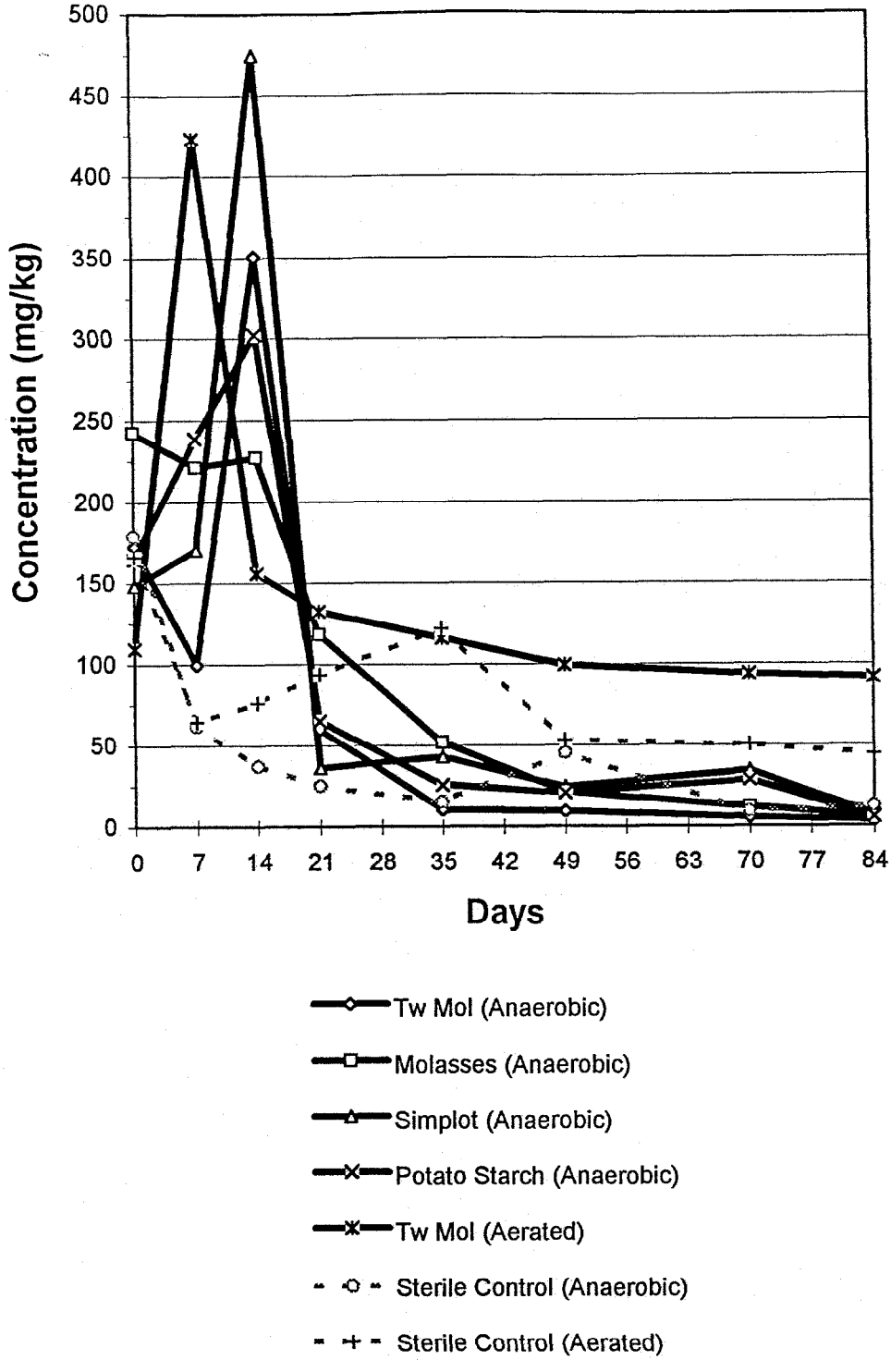


Figure 3-9. Formation and degradation of of total Amino-nitrotoluene compounds in anaerobic and aerated biocell reactors

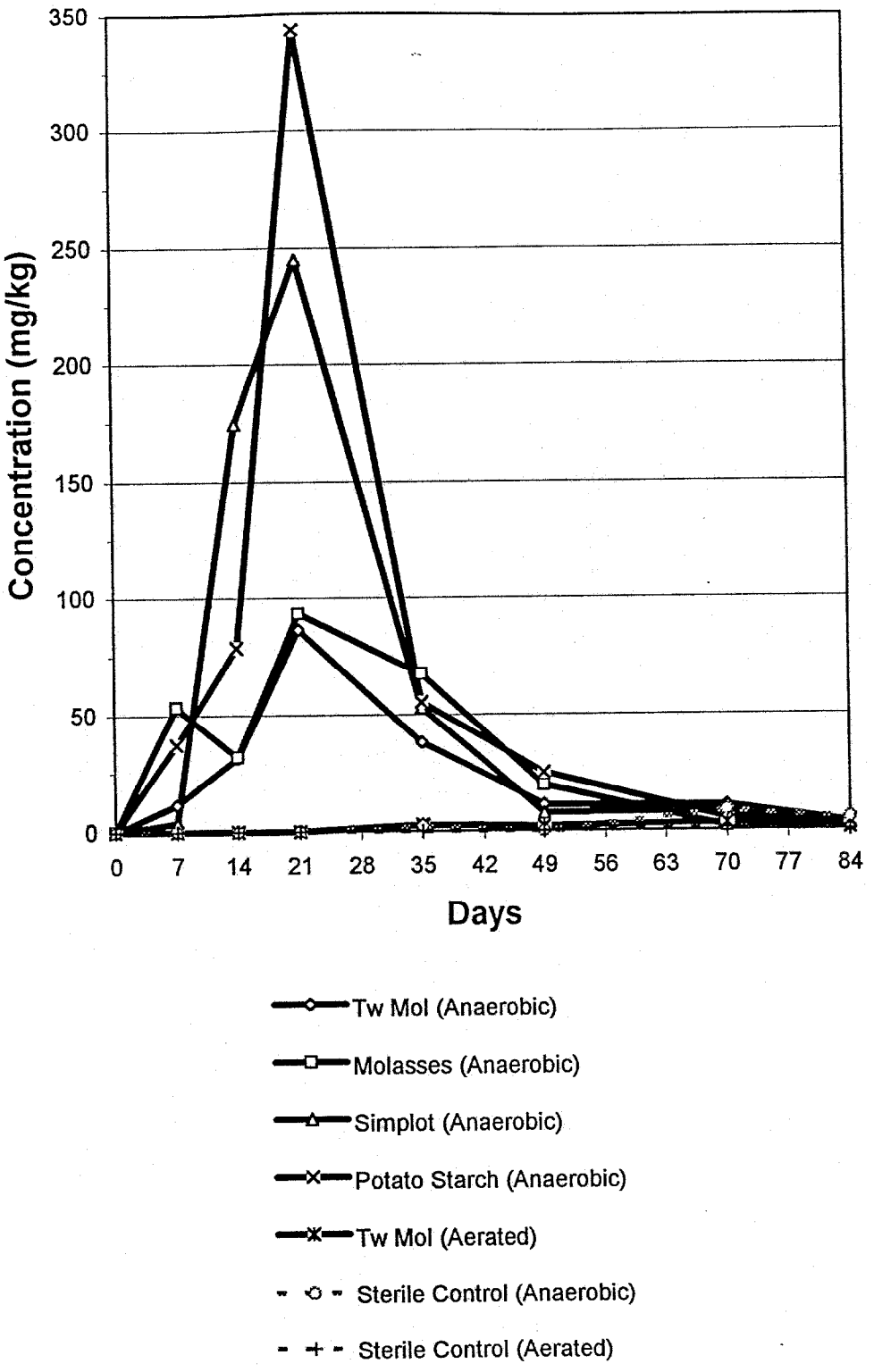


Figure 3-10. Formation and degradation of total di-aminonitrotoluene compounds in anaerobic and aerated biocell reactors

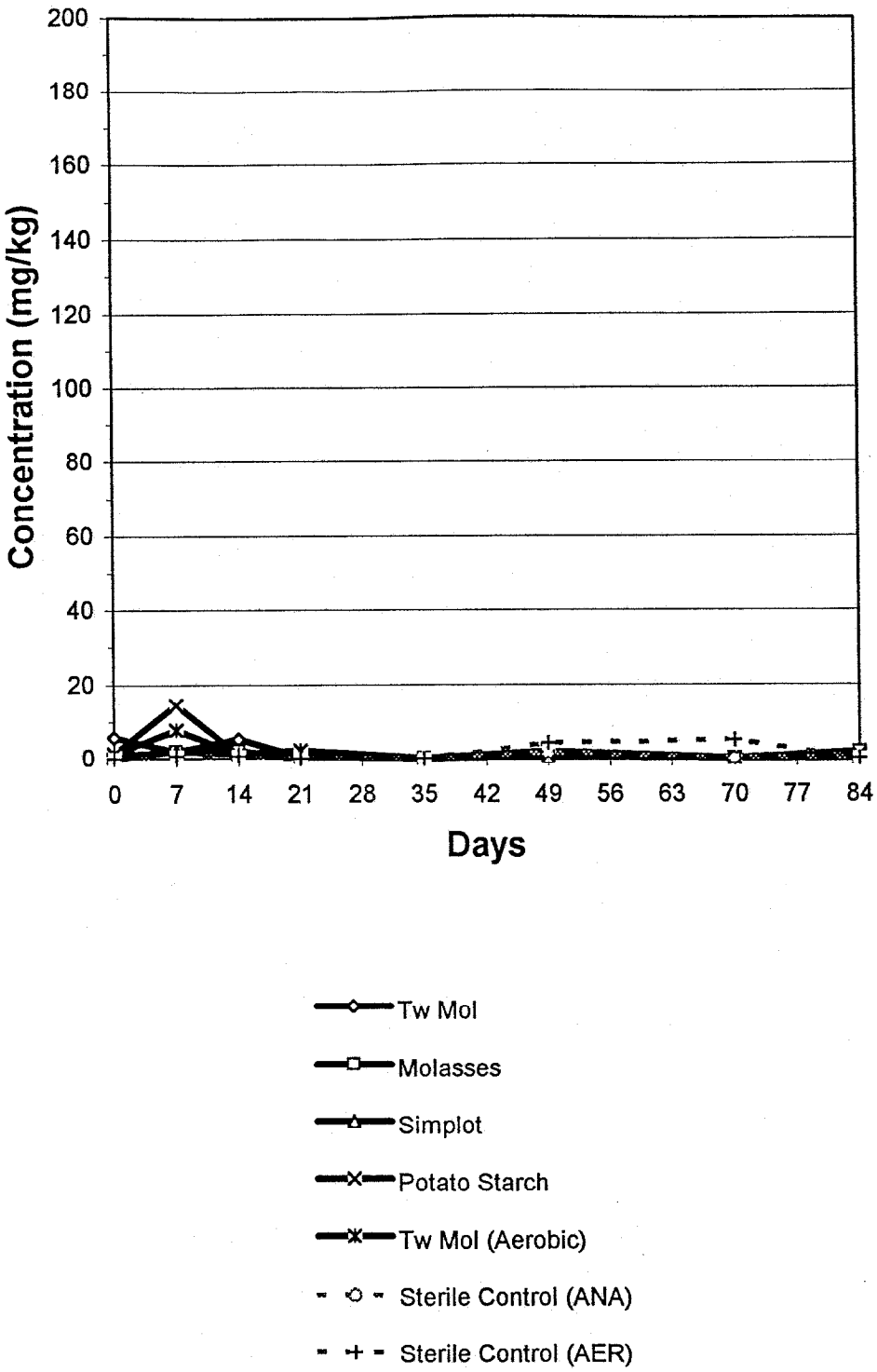


Figure 3-11. Formation and degradation of total azoxy compounds in anaerobic and aerated biocell reactors

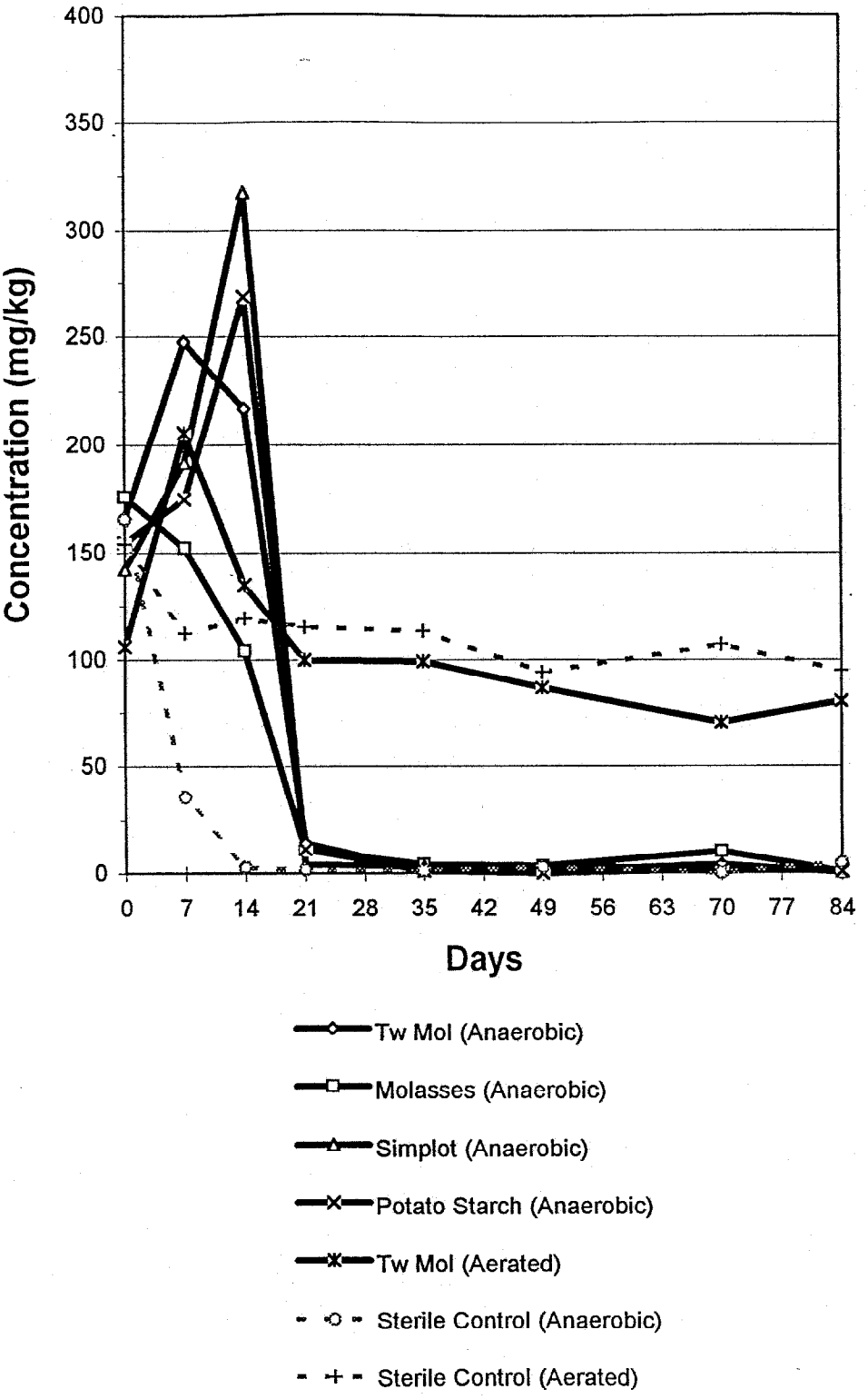


Figure 3-12. Degradation of RDX in anaerobic and aerated biocell reactors

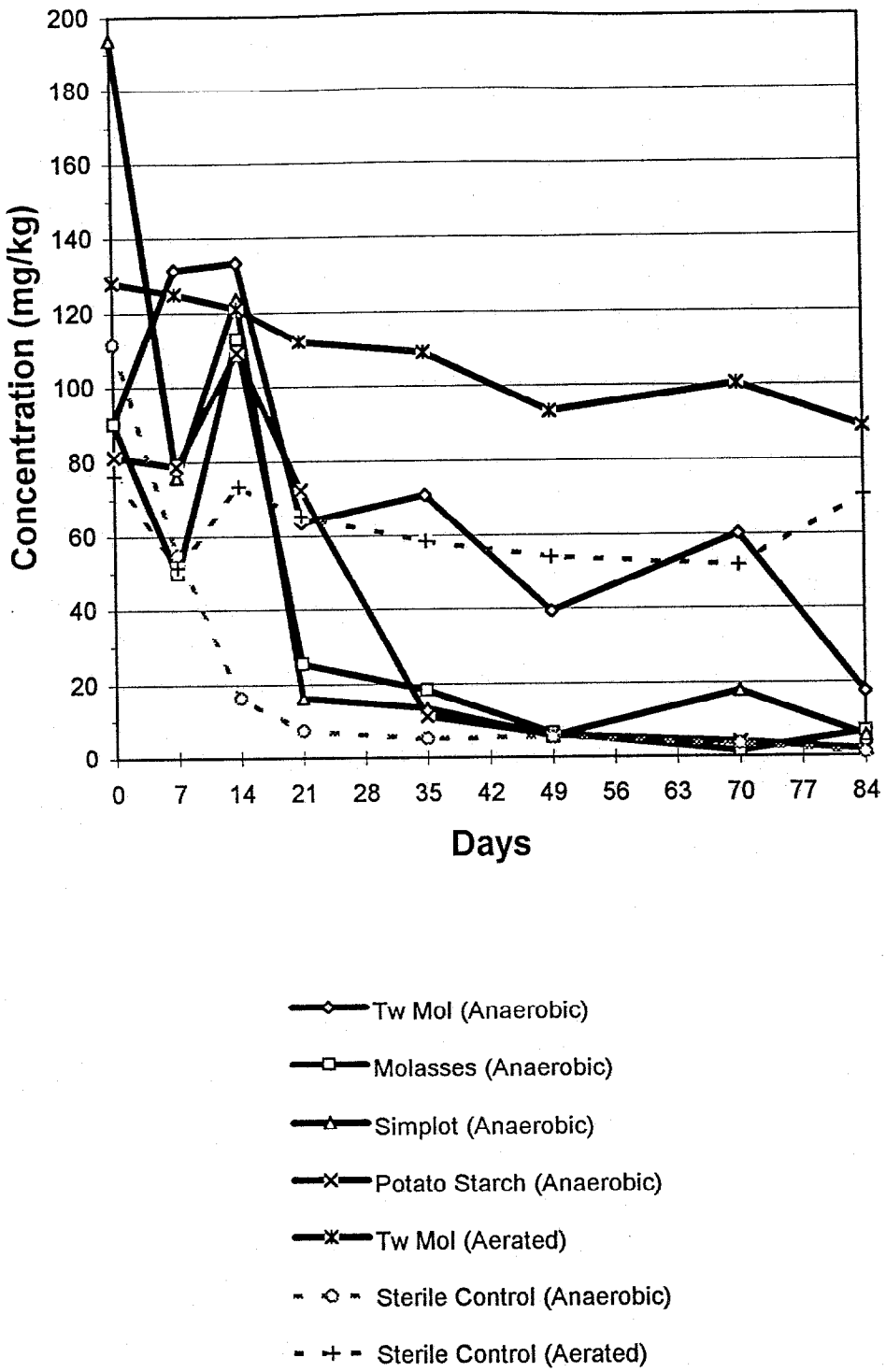


Figure 3-13. Degradation of HMX in anaerobic and aerated biocell reactors

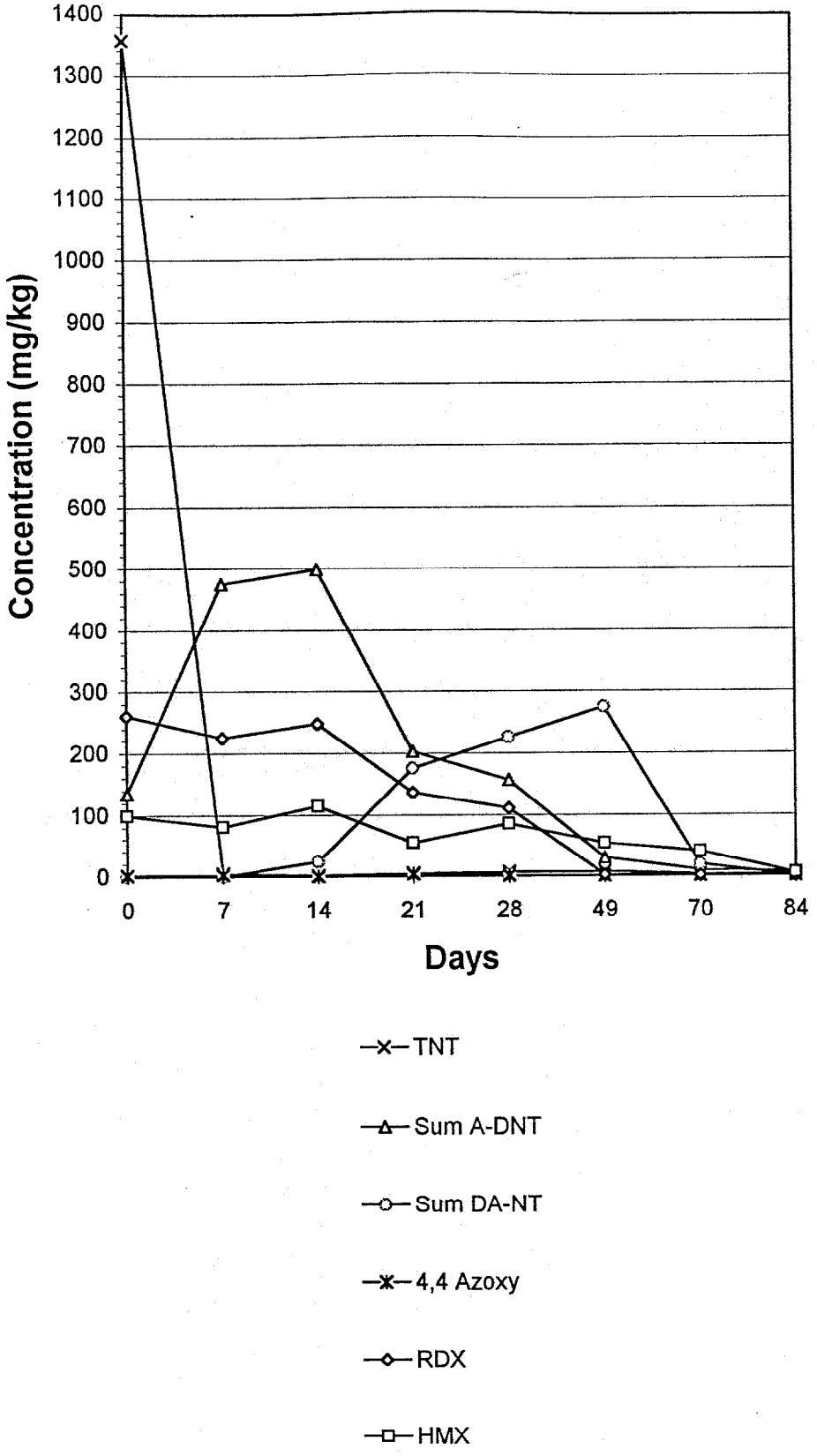


Figure 3-14. Degradation of explosive compounds in aerated Tween 80 and Molasses bioslurry reactors

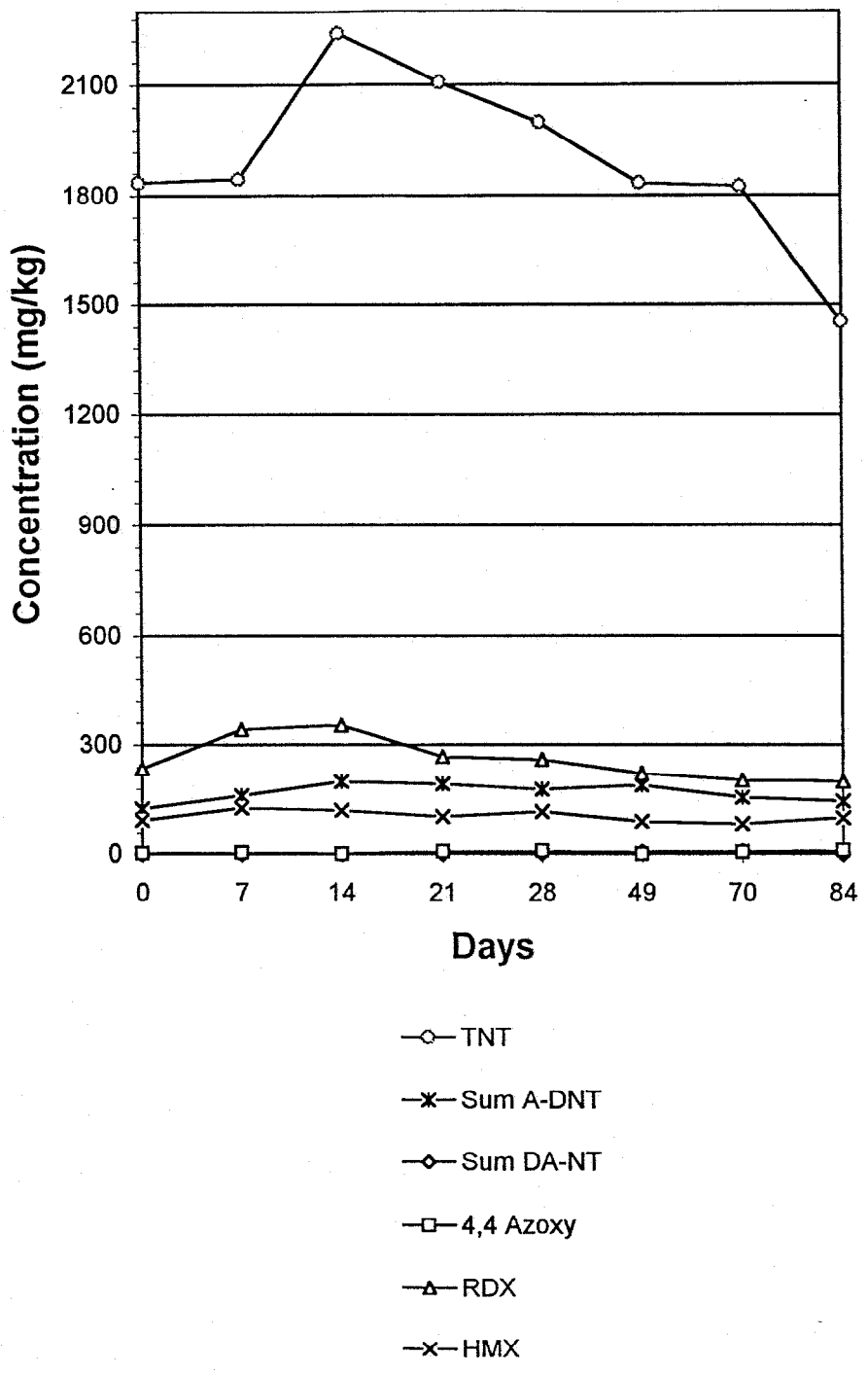


Figure 3-15. Degradation of explosive compounds in aerated No Additives bioslurry reactors

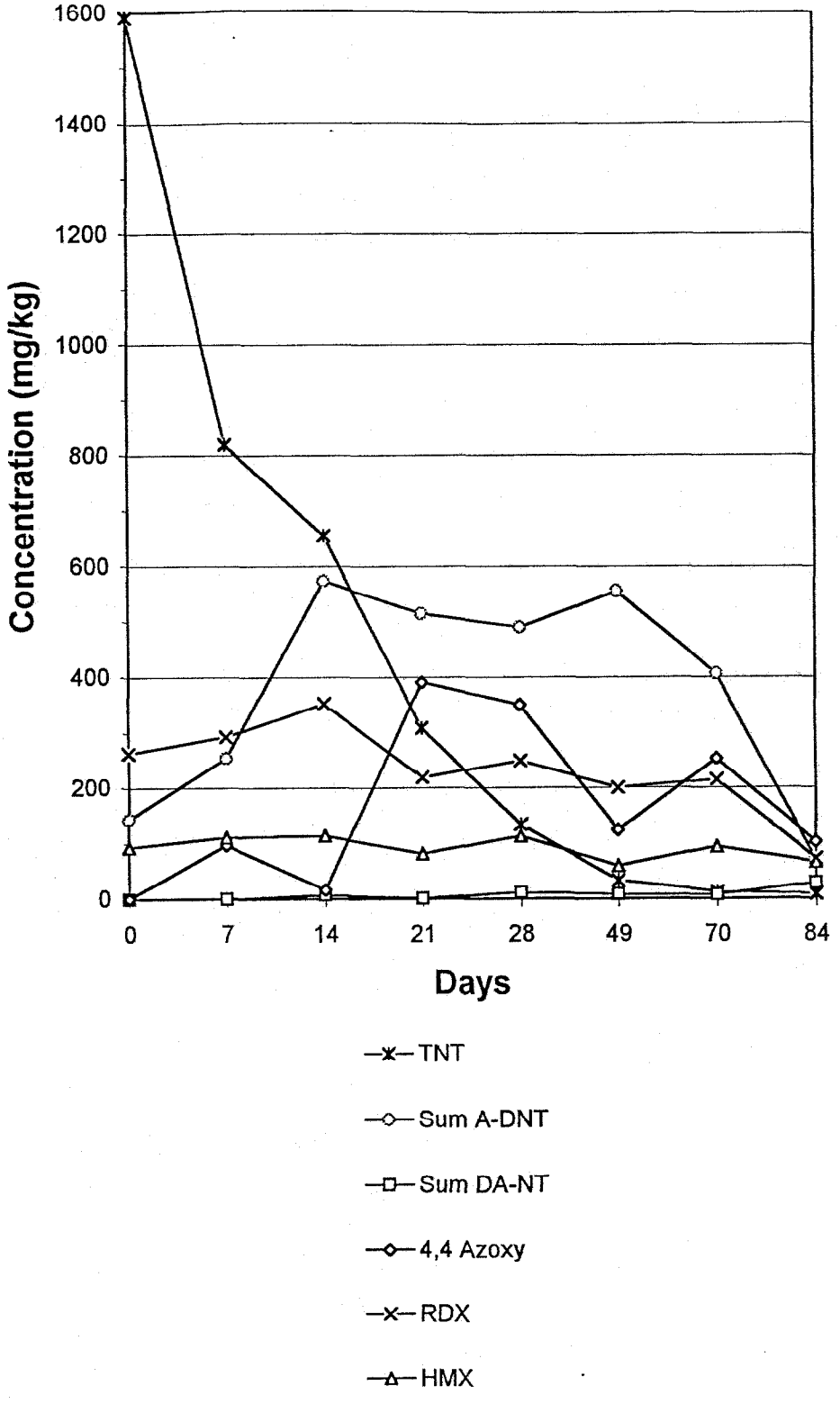


Figure 3-16. Degradation of explosive compounds in anaerobic Simplot bioslurry reactors

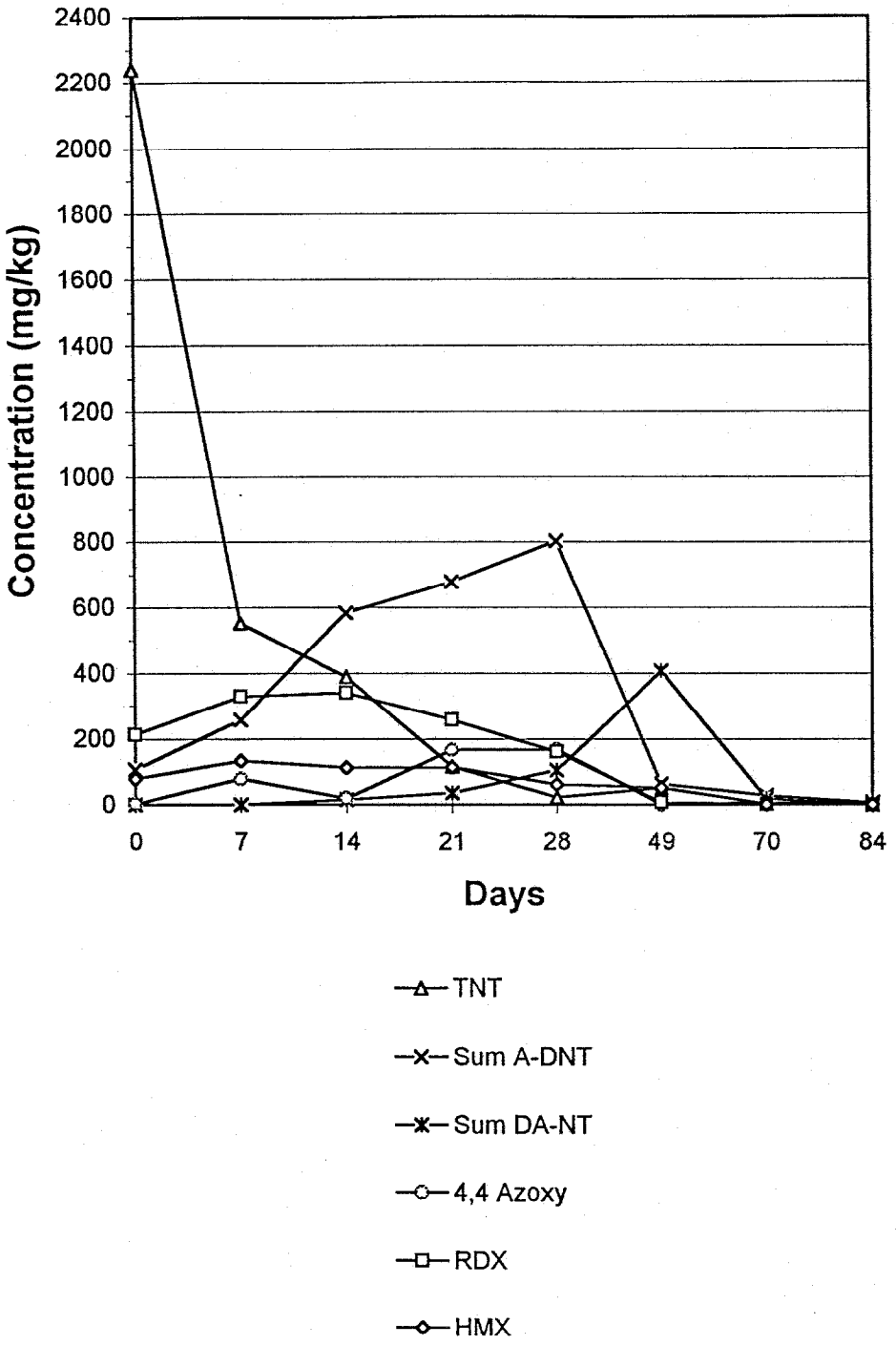


Figure 3-17. Degradation of explosive compounds in anaerobic Simplot with 4hr mix per day bioslurry reactor

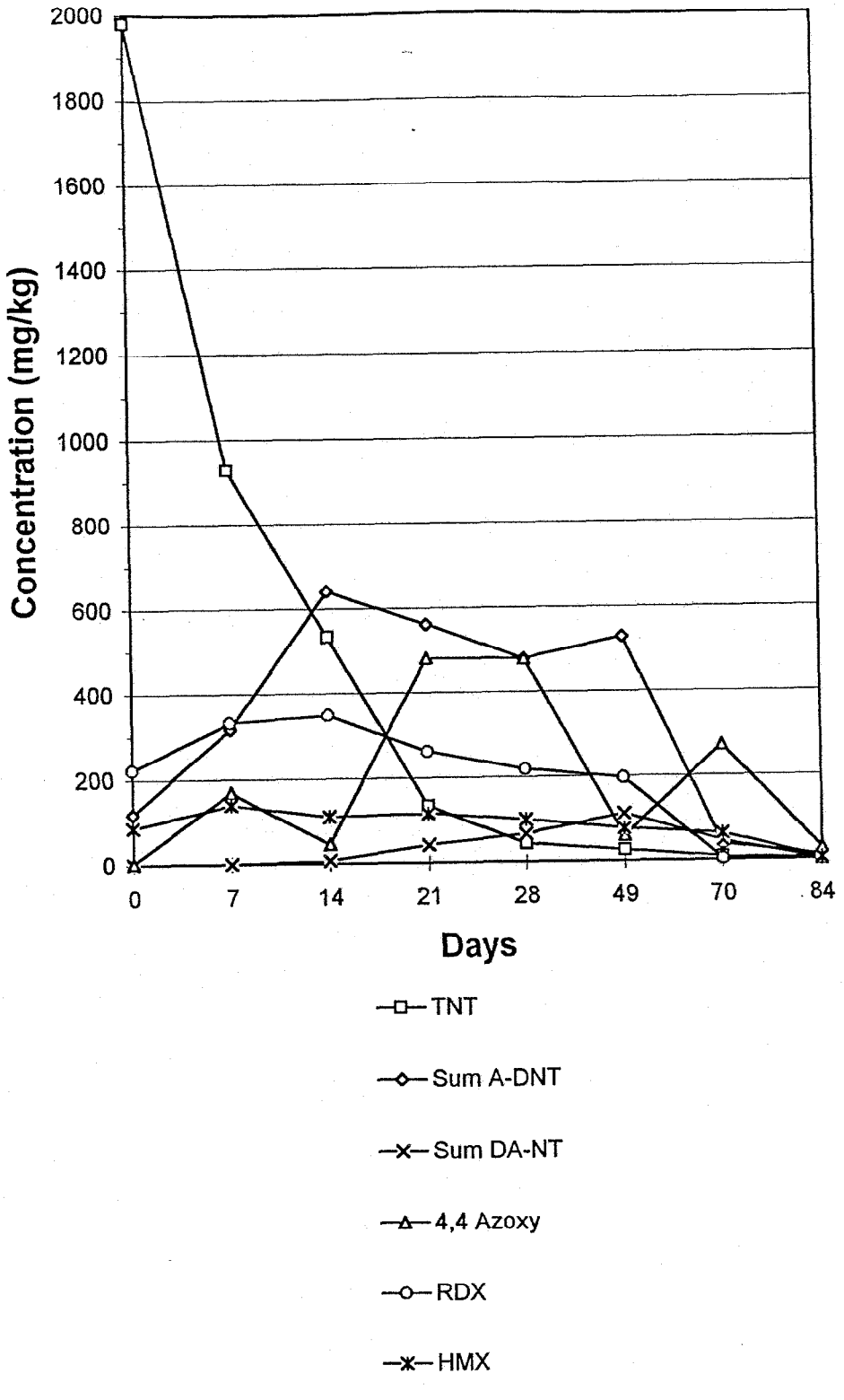


Figure 3-18. Degradation of explosive compounds in anaerobic Potato Starch bioslurry reactors

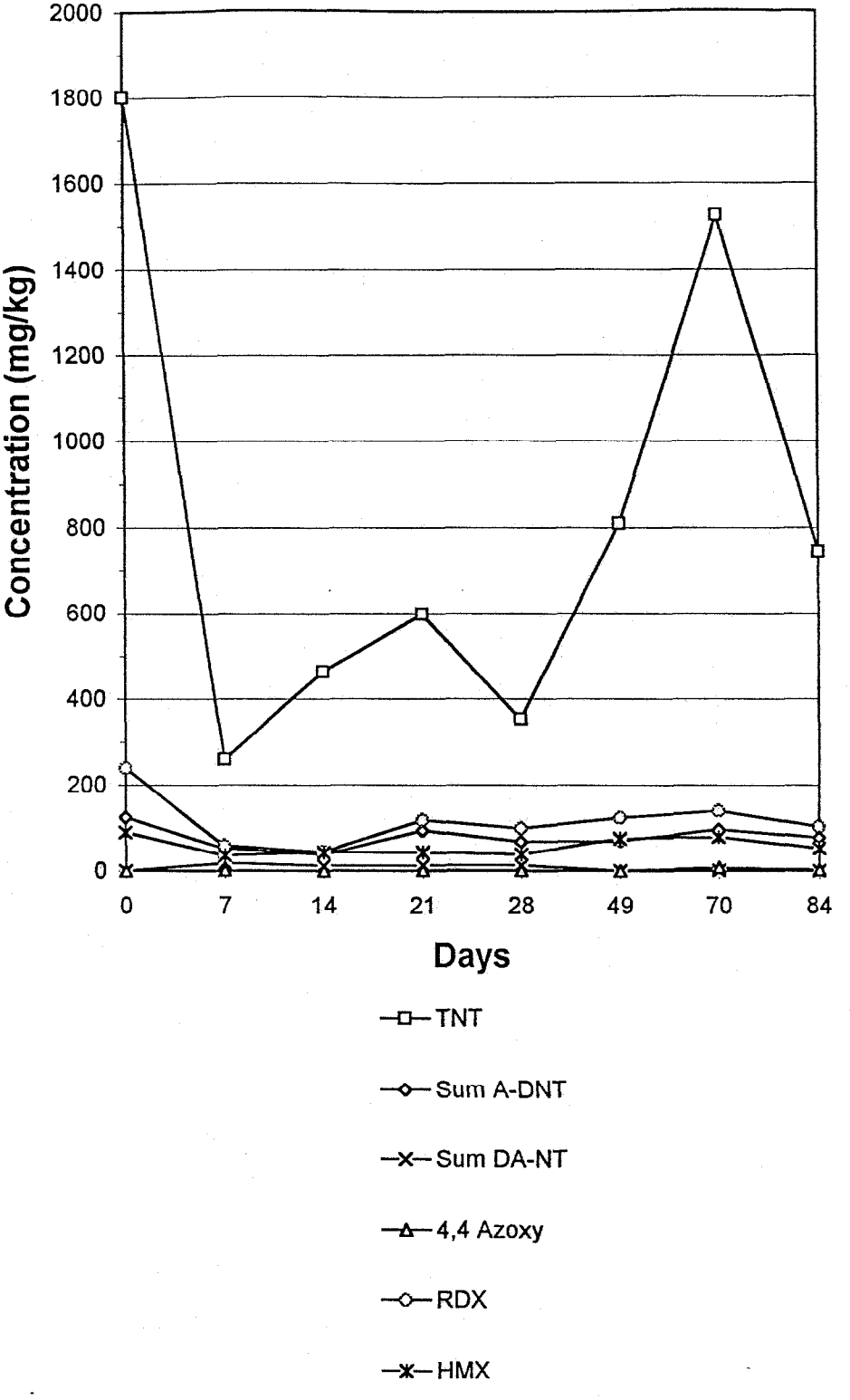


Figure 3-19. Degradation of explosive compounds in non-aerated Sterile Control bioslurry reactors

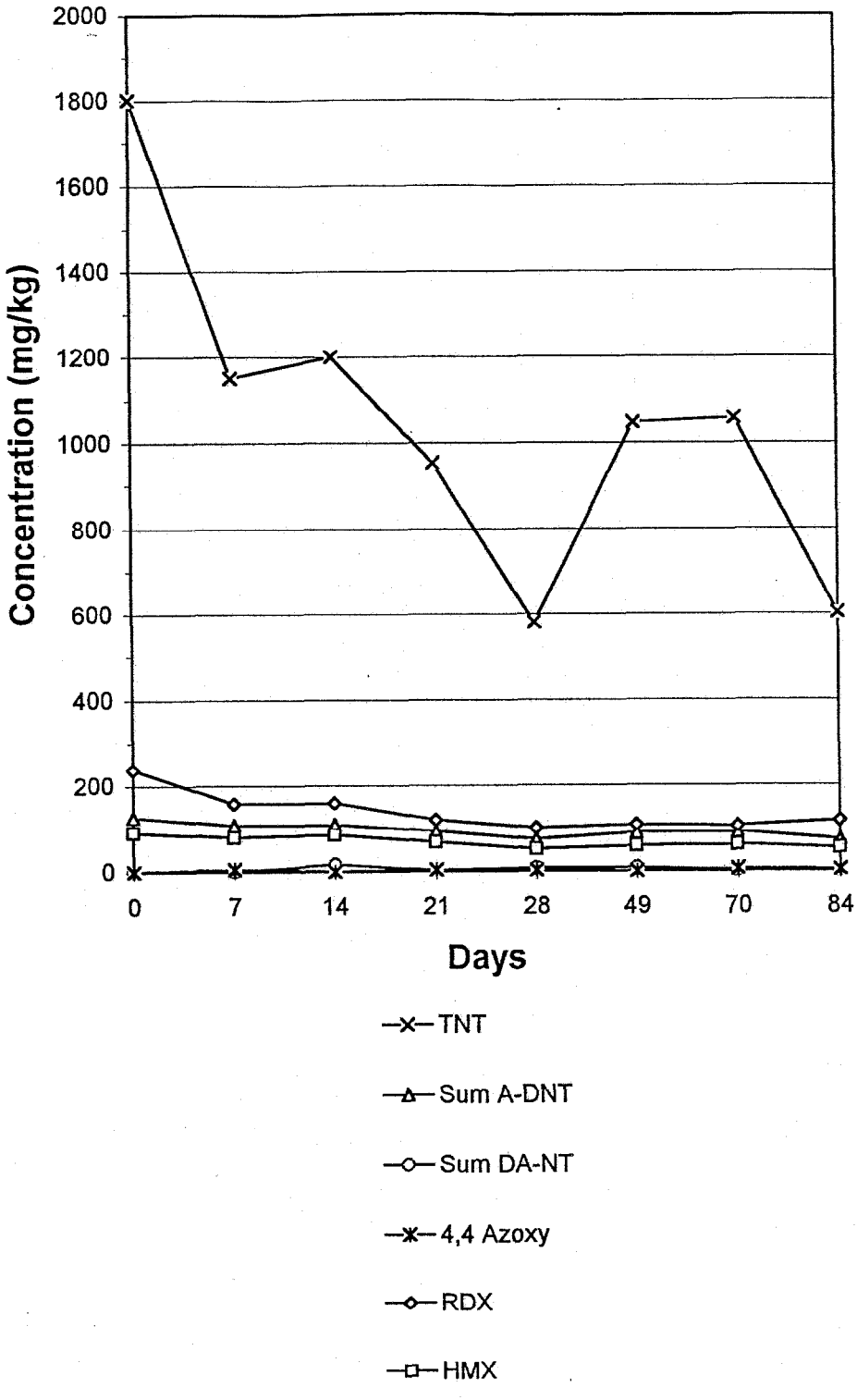


Figure 3-20. Concentration of explosive compounds in aerated Sterile Control bioslurry reactors

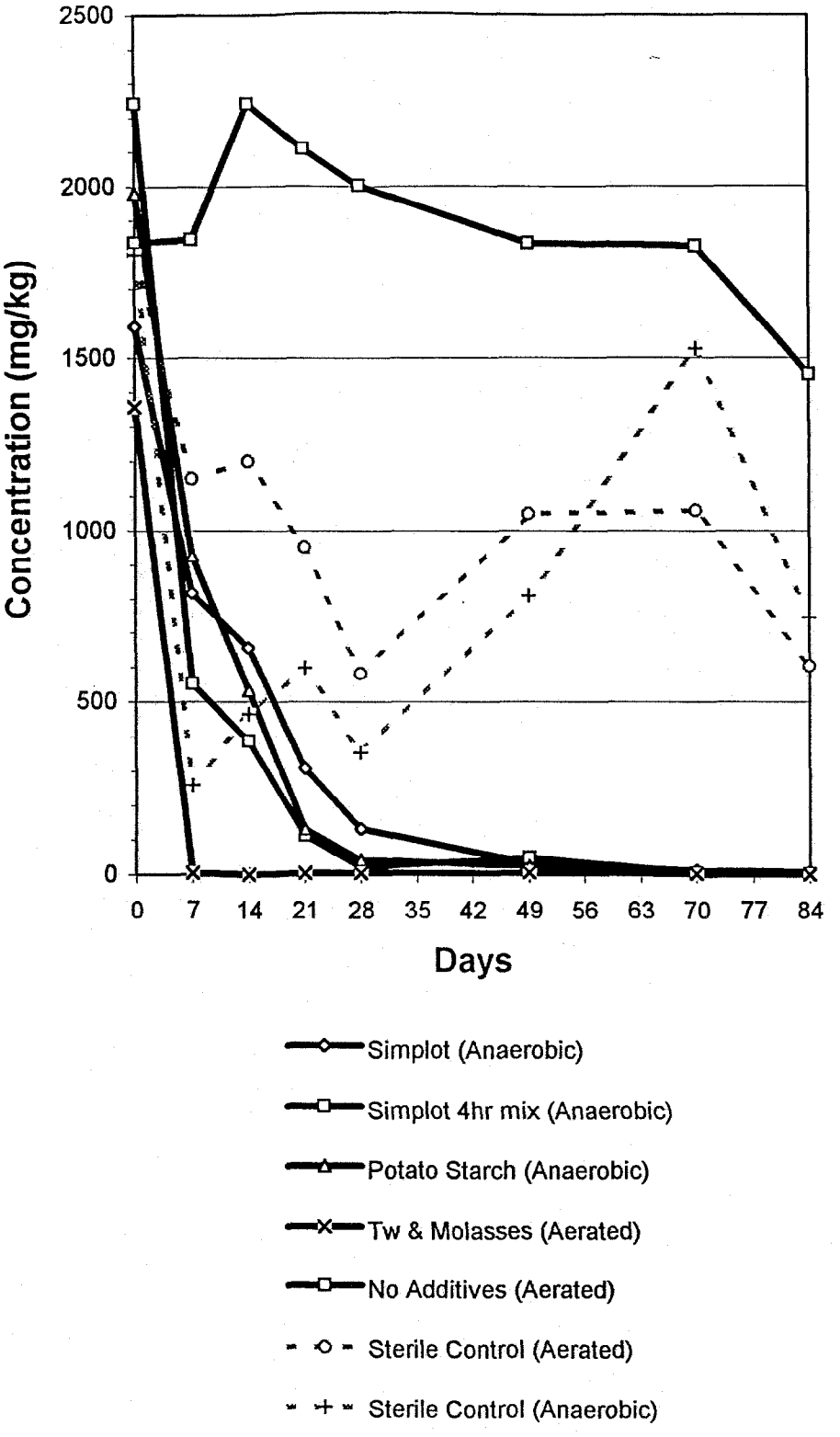


Figure 3-21. Degradation of TNT in anaerobic and aerated bioslurry reactors

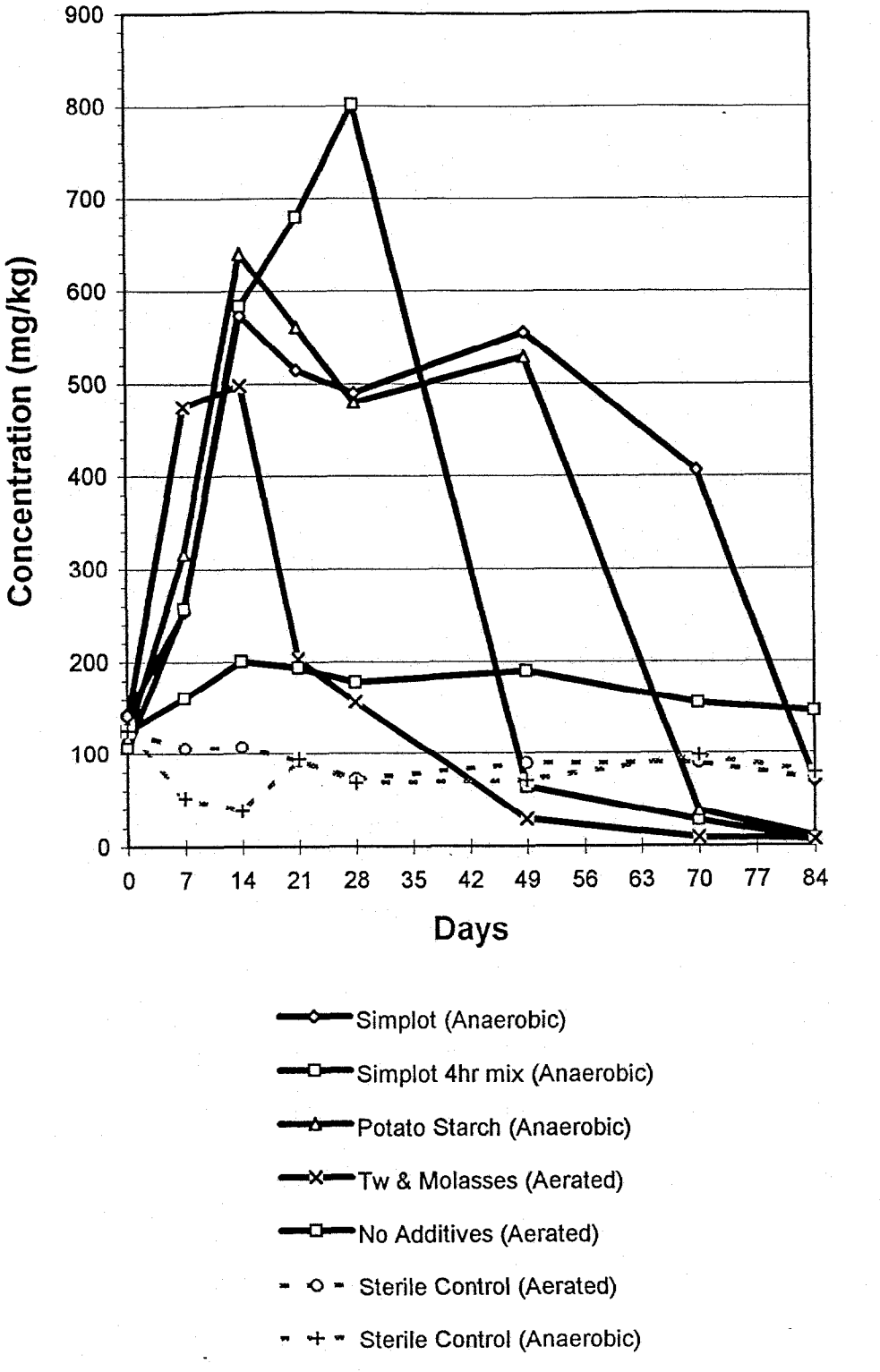


Figure 3-22. Formation and degradation of total amino-dinitrotoluene compounds in anaerobic and aerated bioslurry reactors.

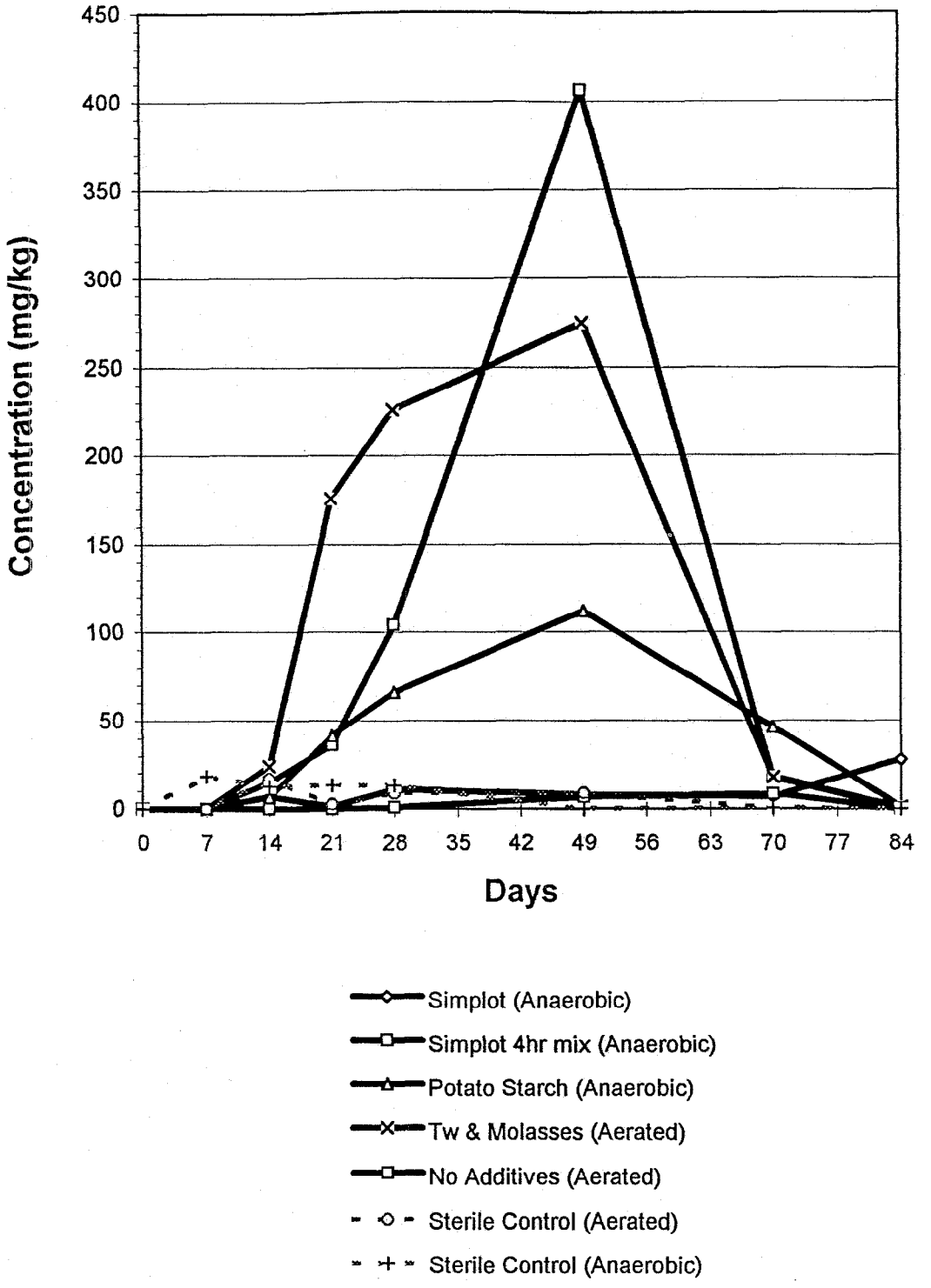


Figure 3-23. Formation and degradation of total diamino-nitrotoluene compounds in anaerobic and aerated bioslurry reactors.

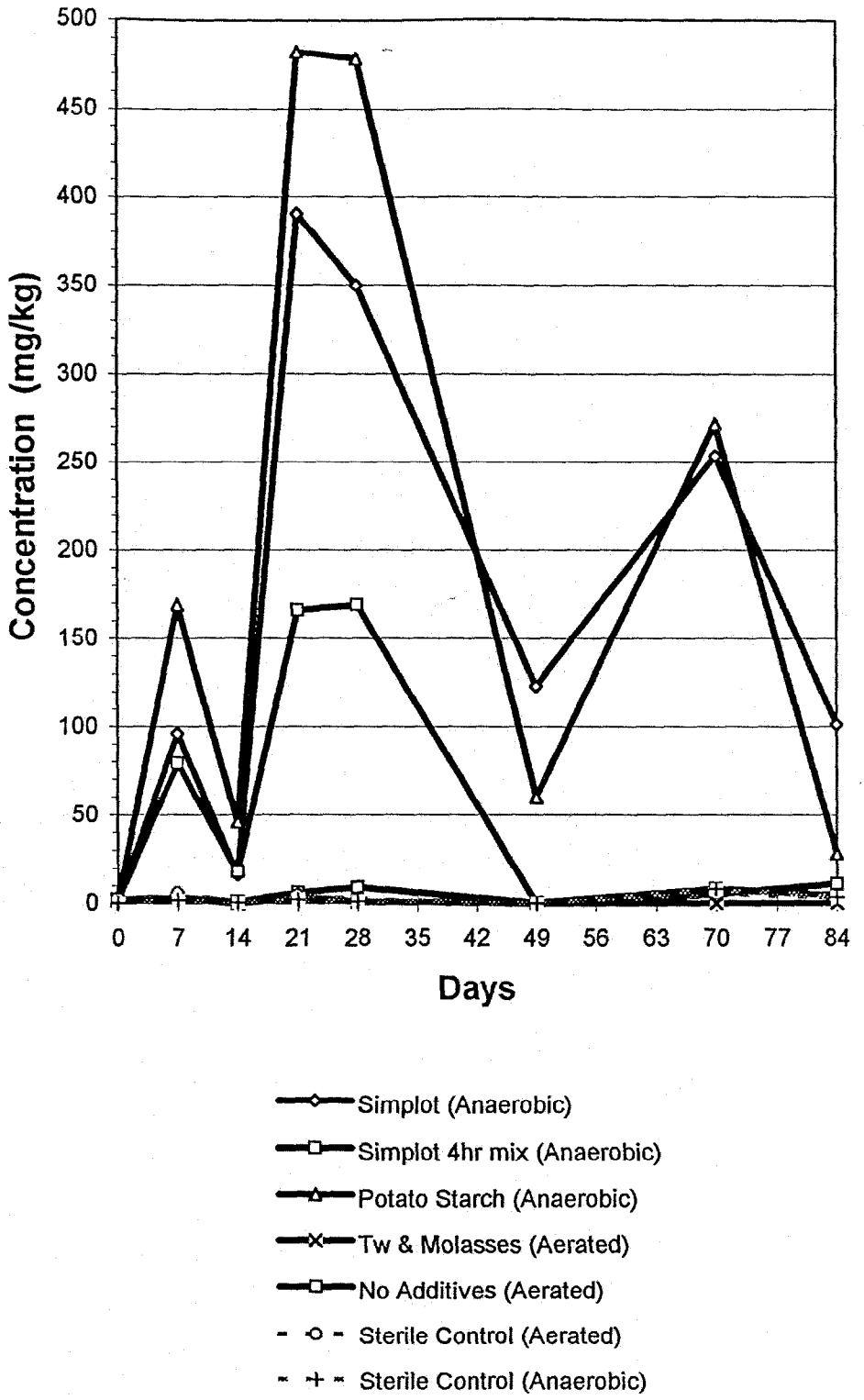


Figure 3-24. Formation and degradation of total azoxy compounds in anaerobic and aerated bioslurry reactors.

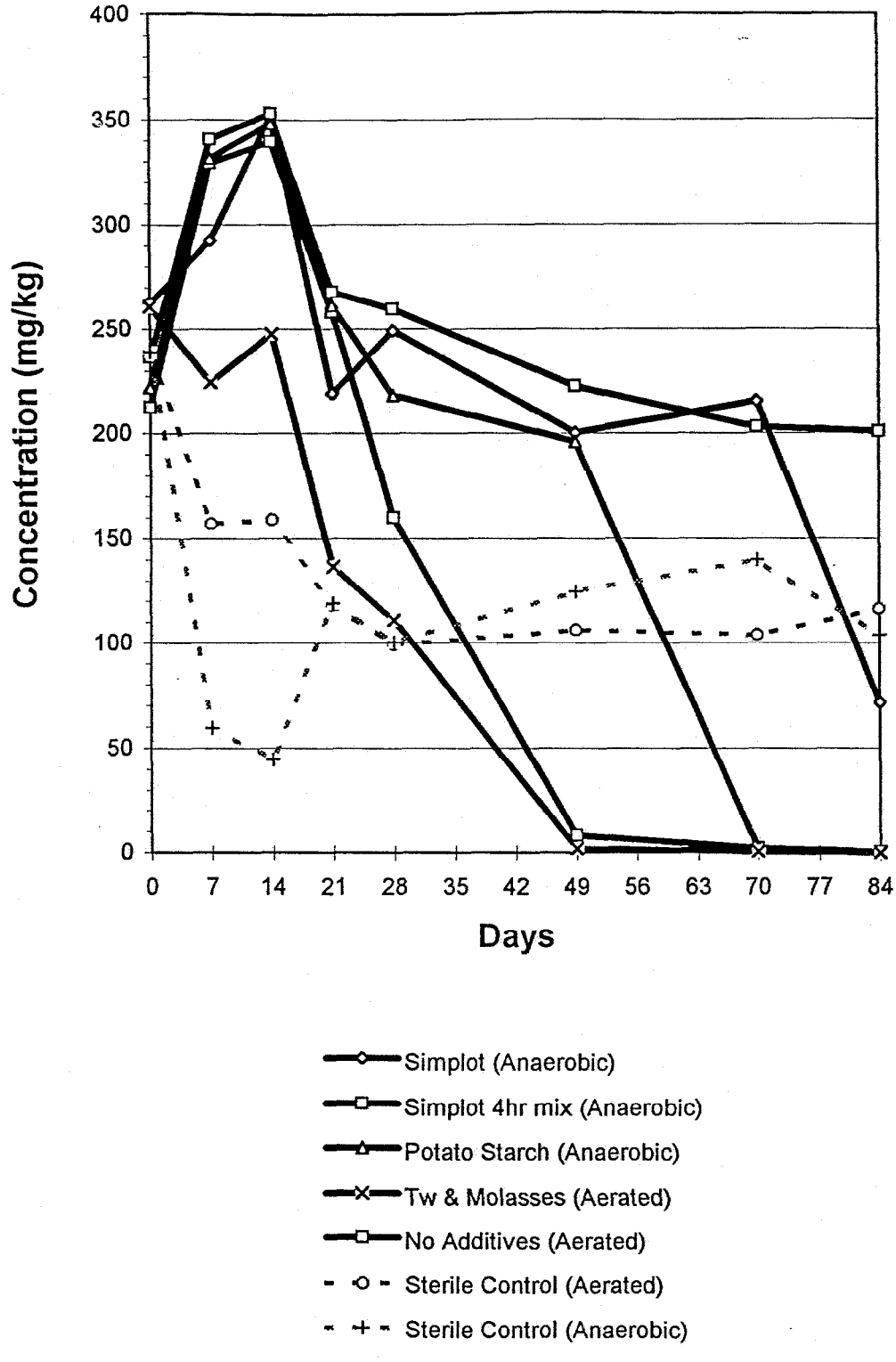


Figure 3-25. Degradation of RDX in anaerobic and aerated bioslurry reactors.

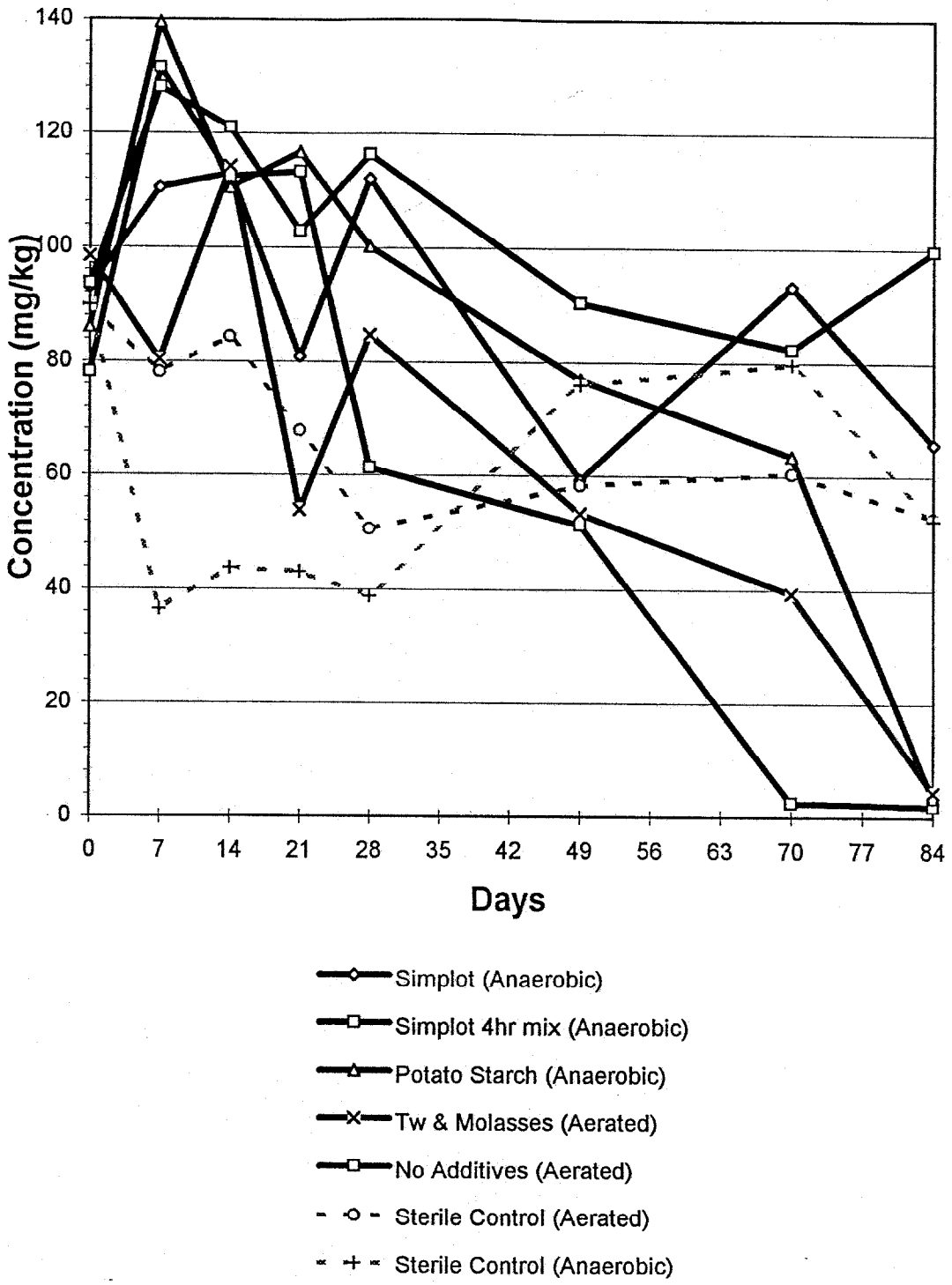


Figure 3-26. Degradation of HMX in anaerobic and aerated bioslurry reactors.

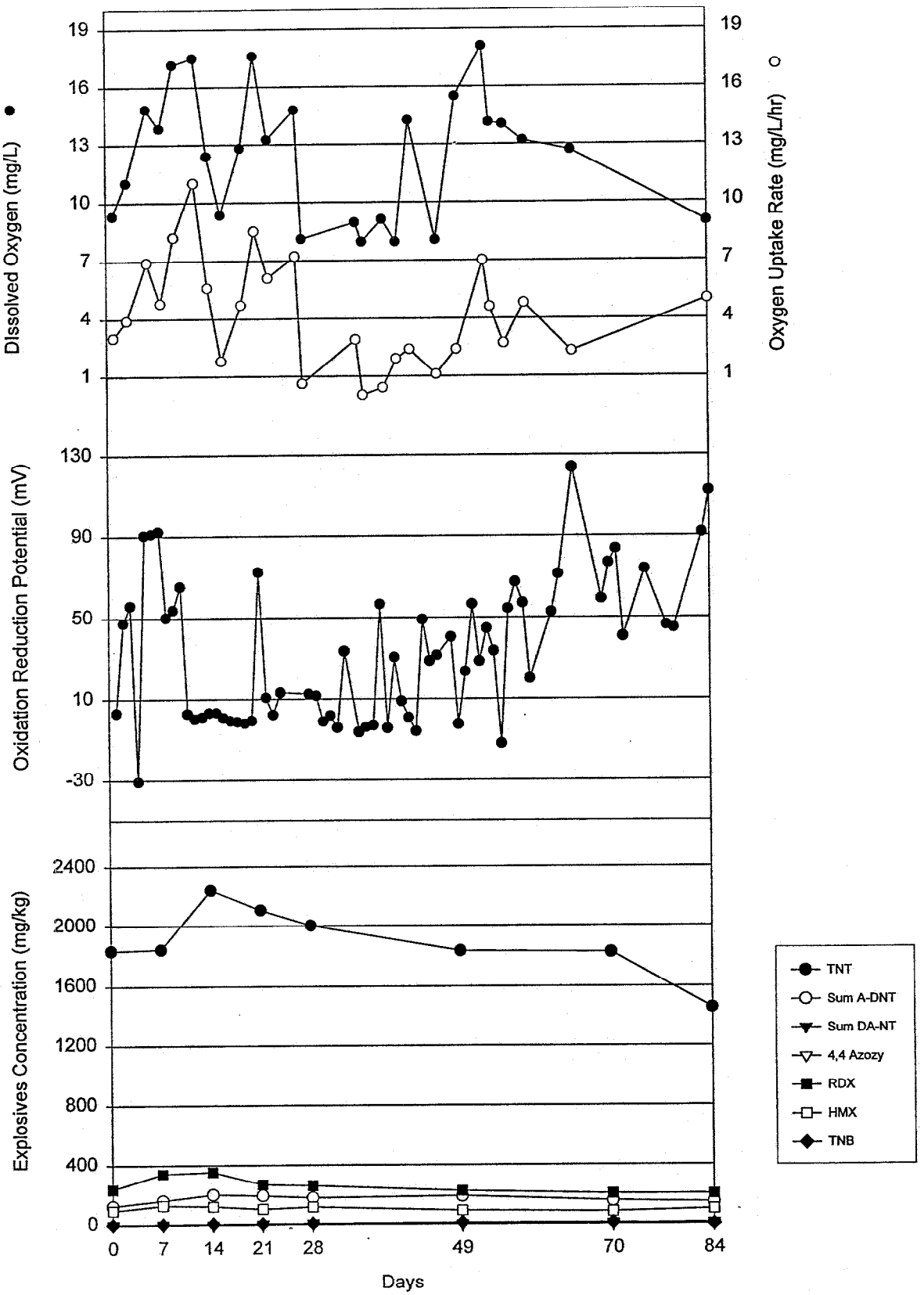


Figure 3-27 Plots of dissolved oxygen, oxygen uptake rate, oxidation reduction potential, and explosives concentration versus time for the aerated bioslurry No Additives treatment

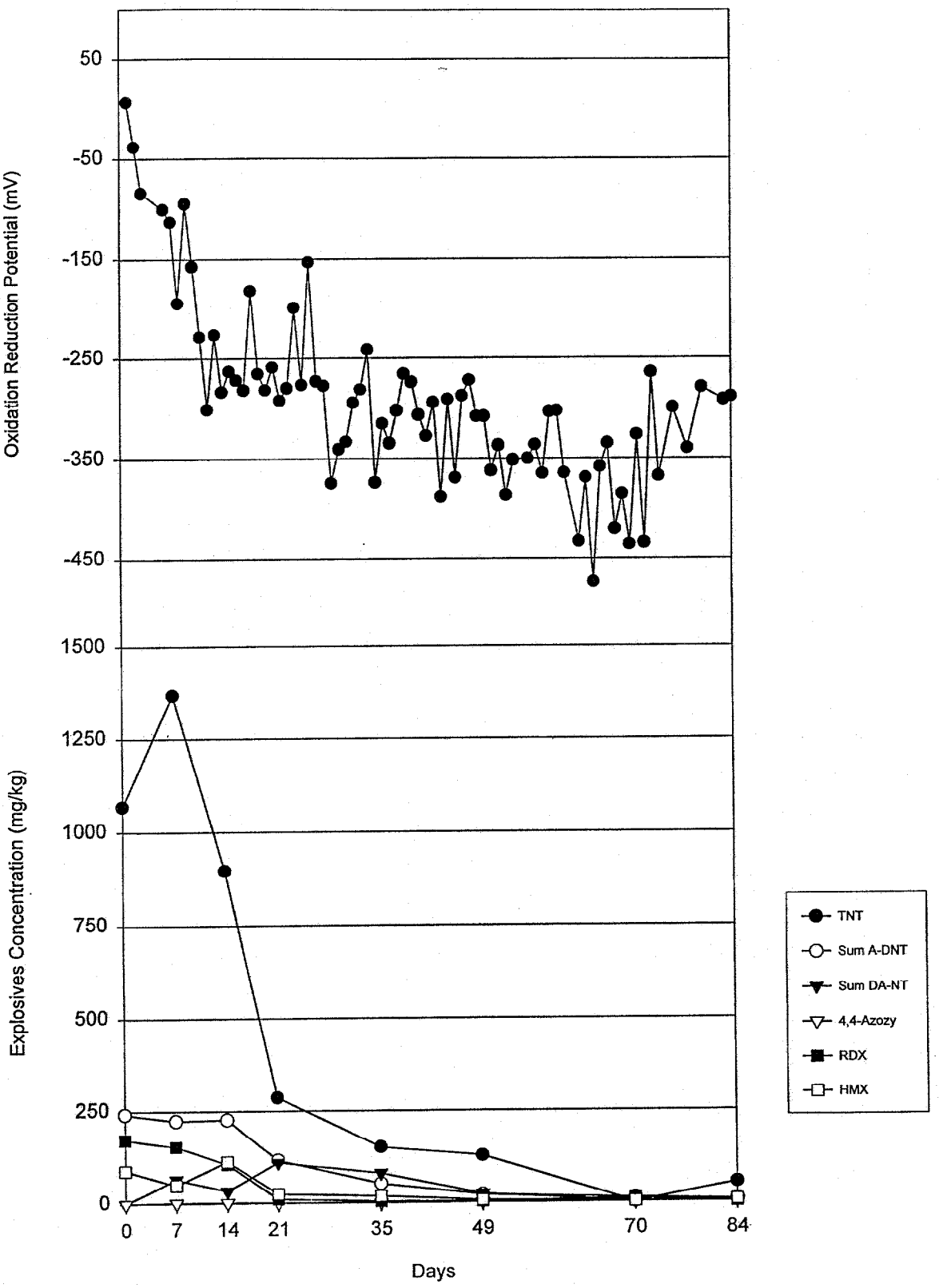


Figure 3-28 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic biocell Molasses treatment

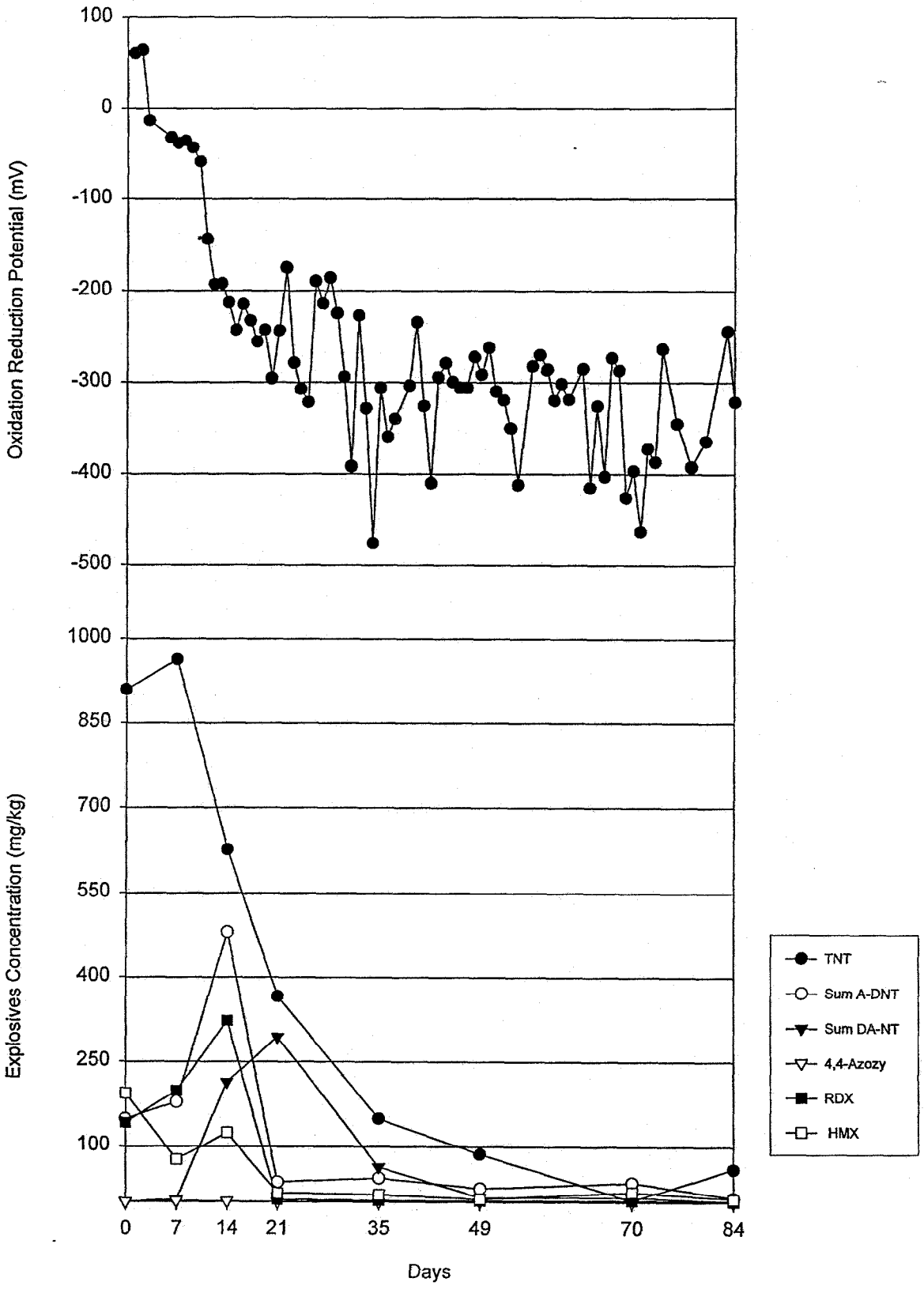


Figure 3-29 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic biocell Simplot treatment

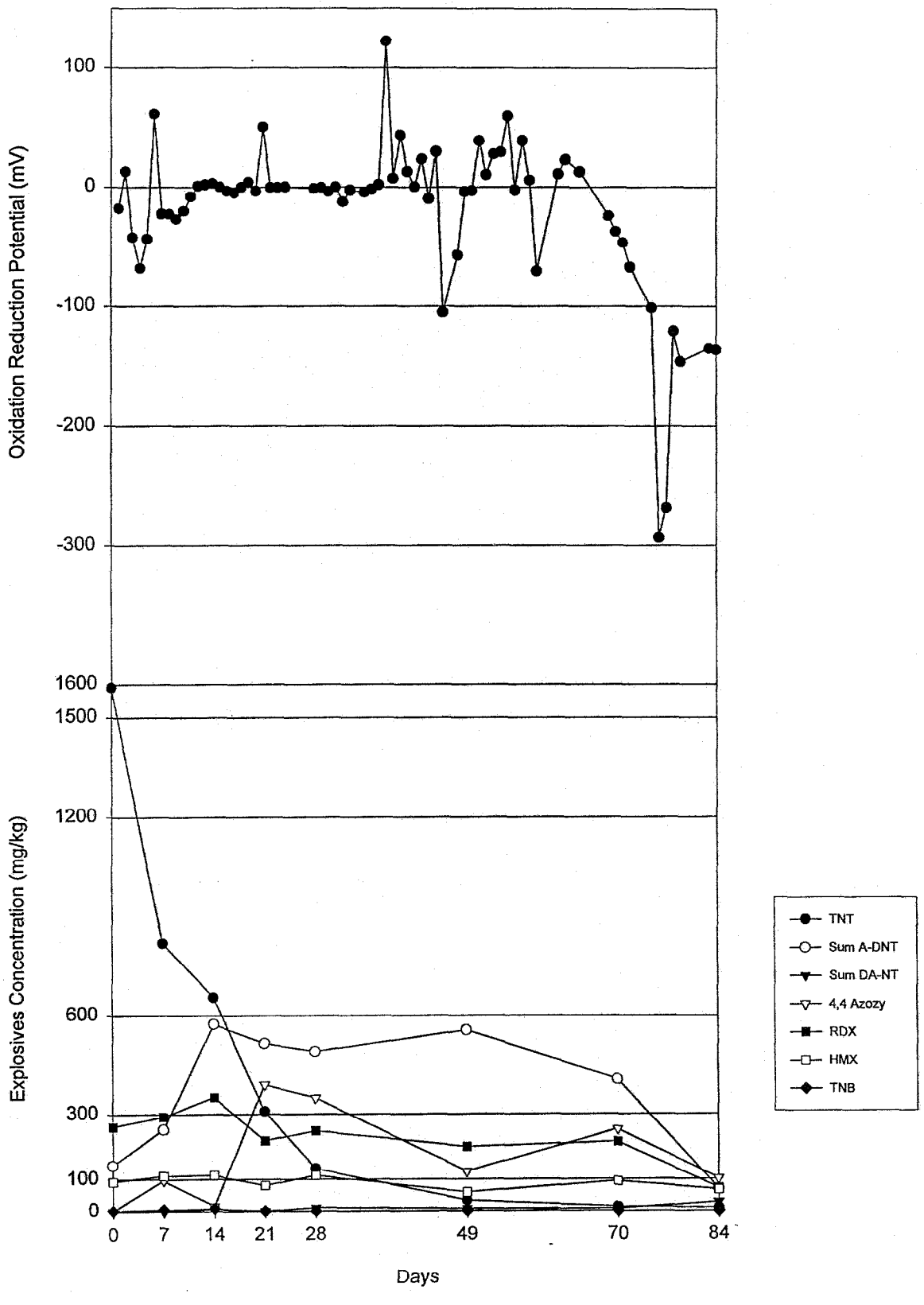


Figure 3-30 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic bioslurry Simplot treatment

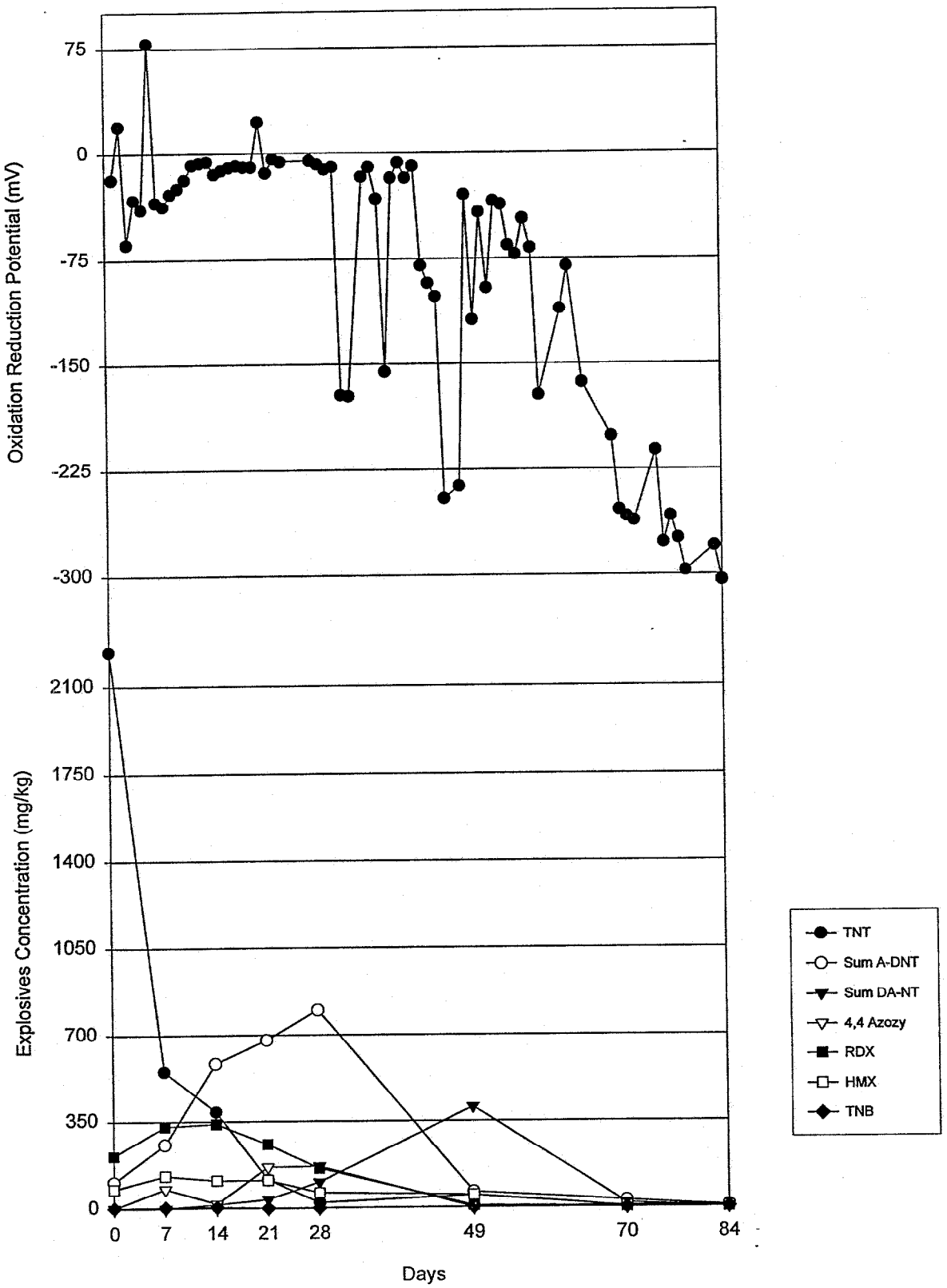


Figure 3-31 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic bioslurry Simplot 4 hours mix per day treatment

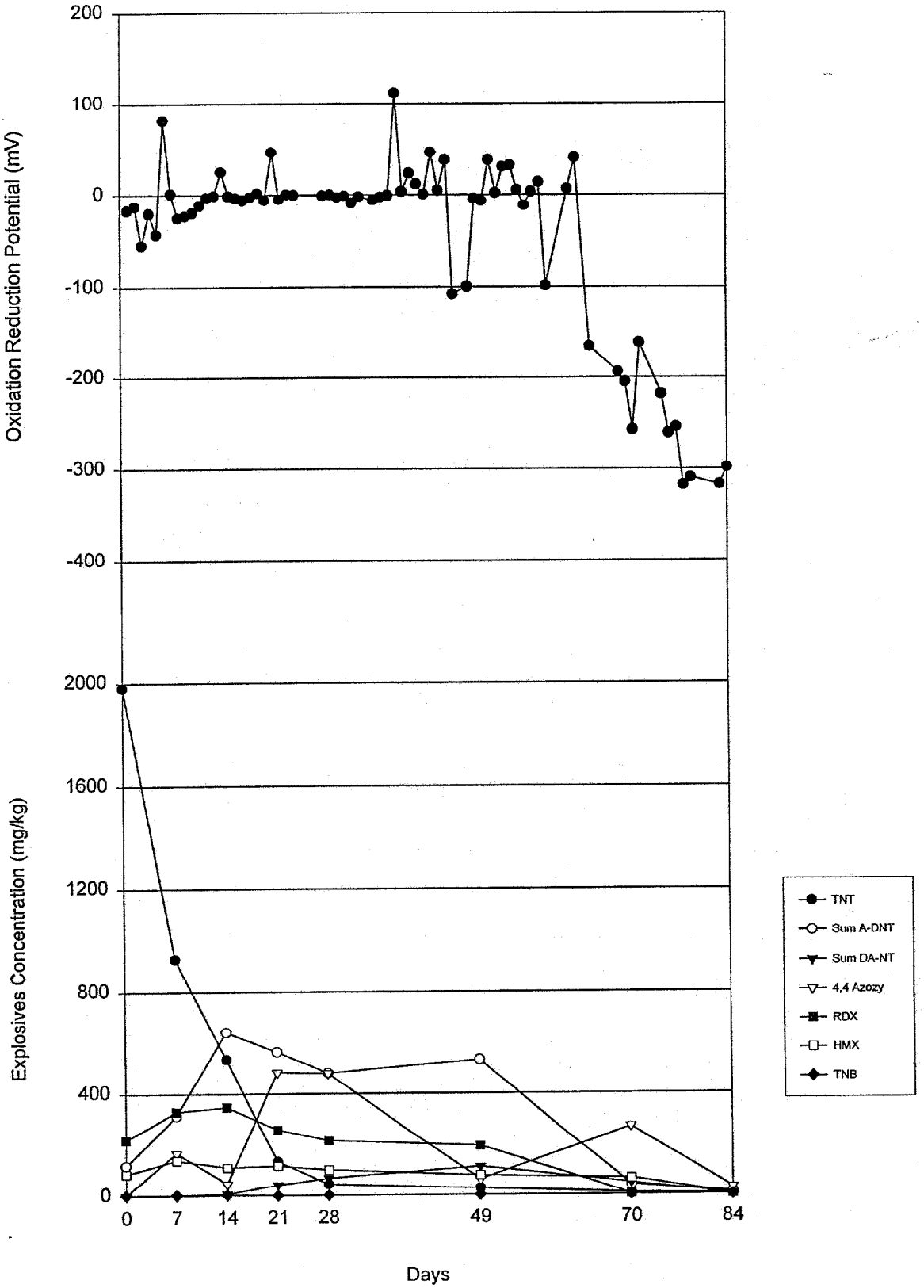


Figure 3-32 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic bioslurry Potato Starch treatment

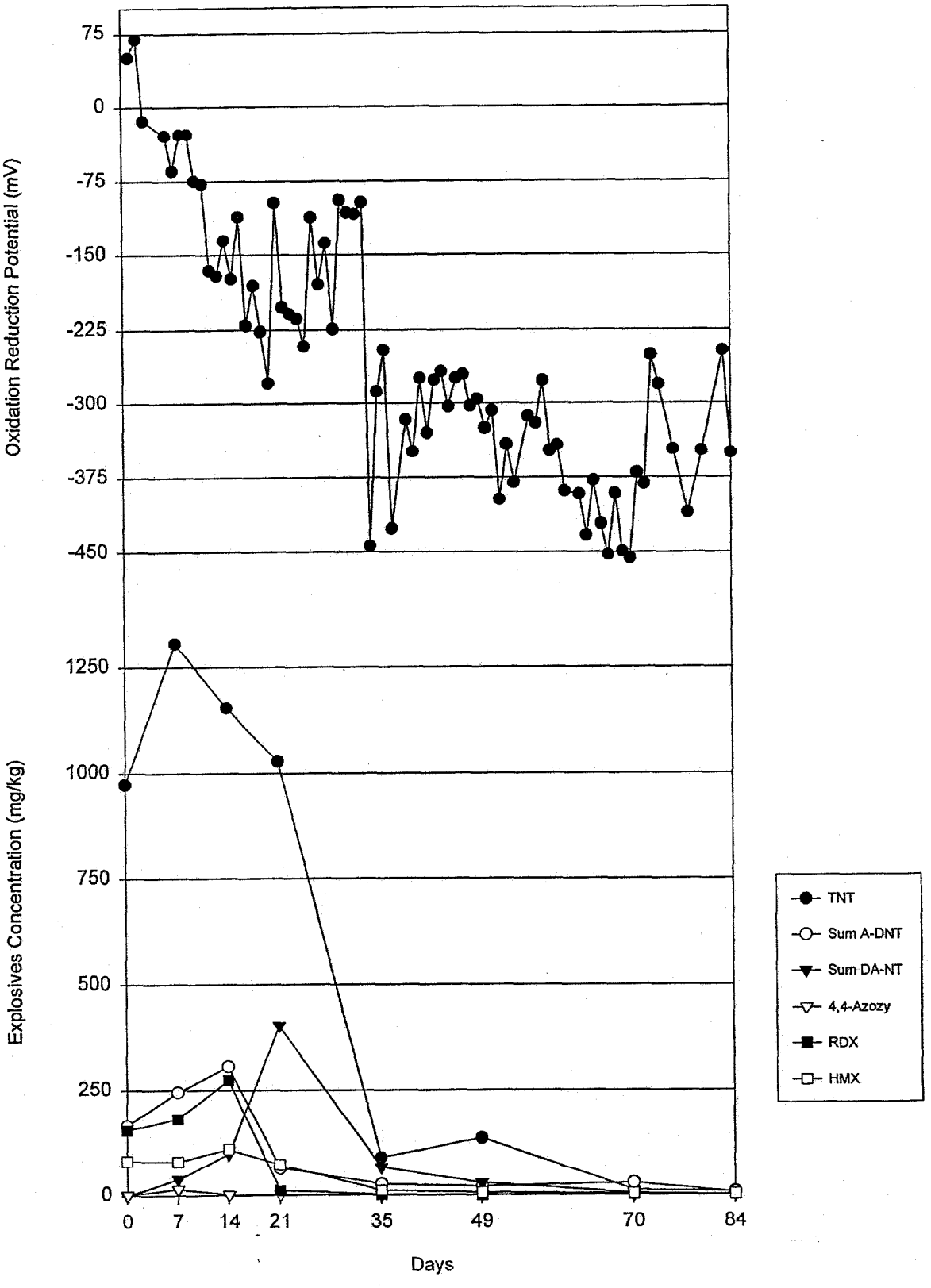


Figure 3-33 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic biocell Potato Starch treatment

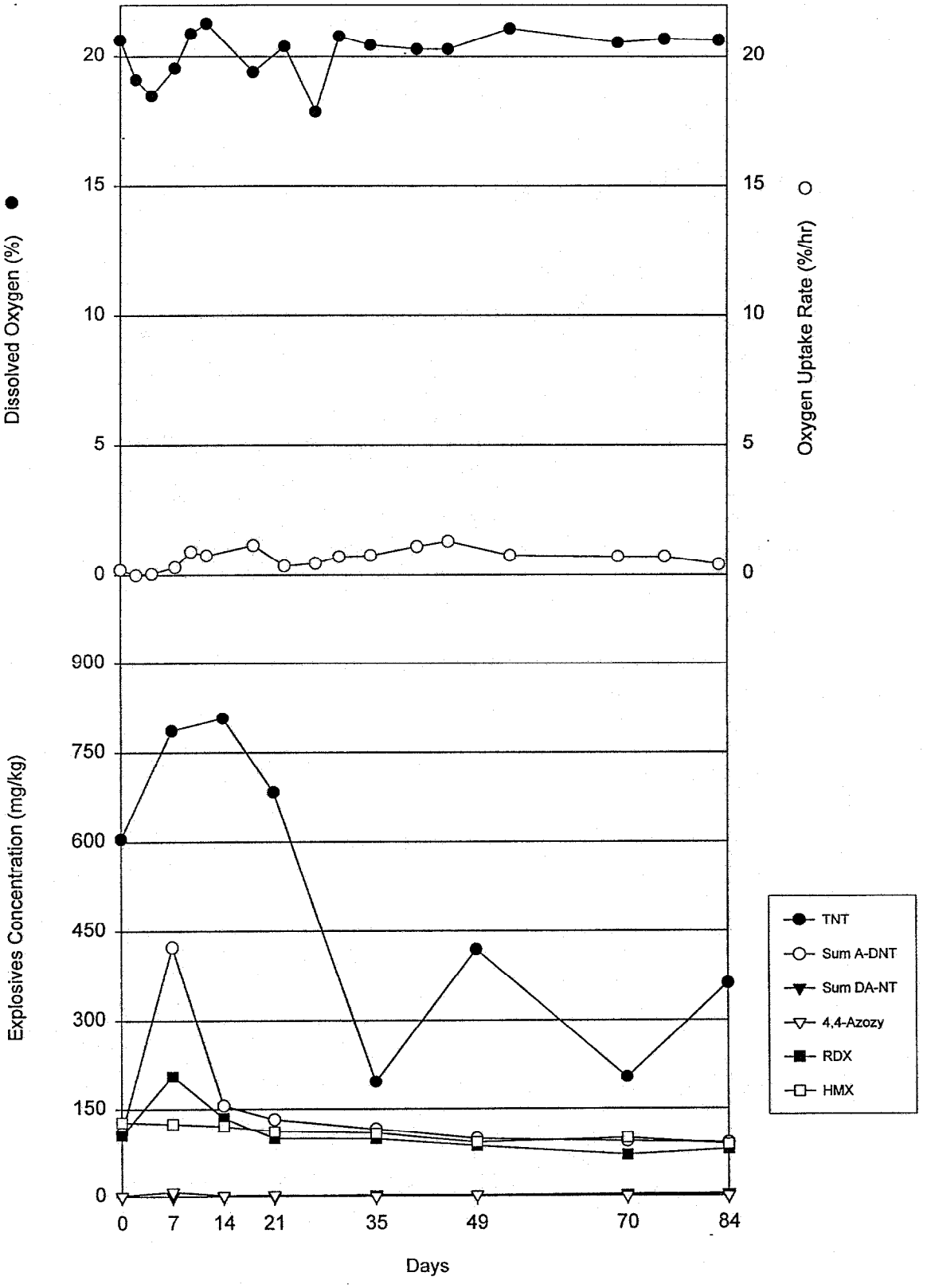


Figure 3-34 Plots of dissolved oxygen, oxygen uptake rate, and explosives concentration versus time for the aerated biocell Tween 80 and Molasses treatment

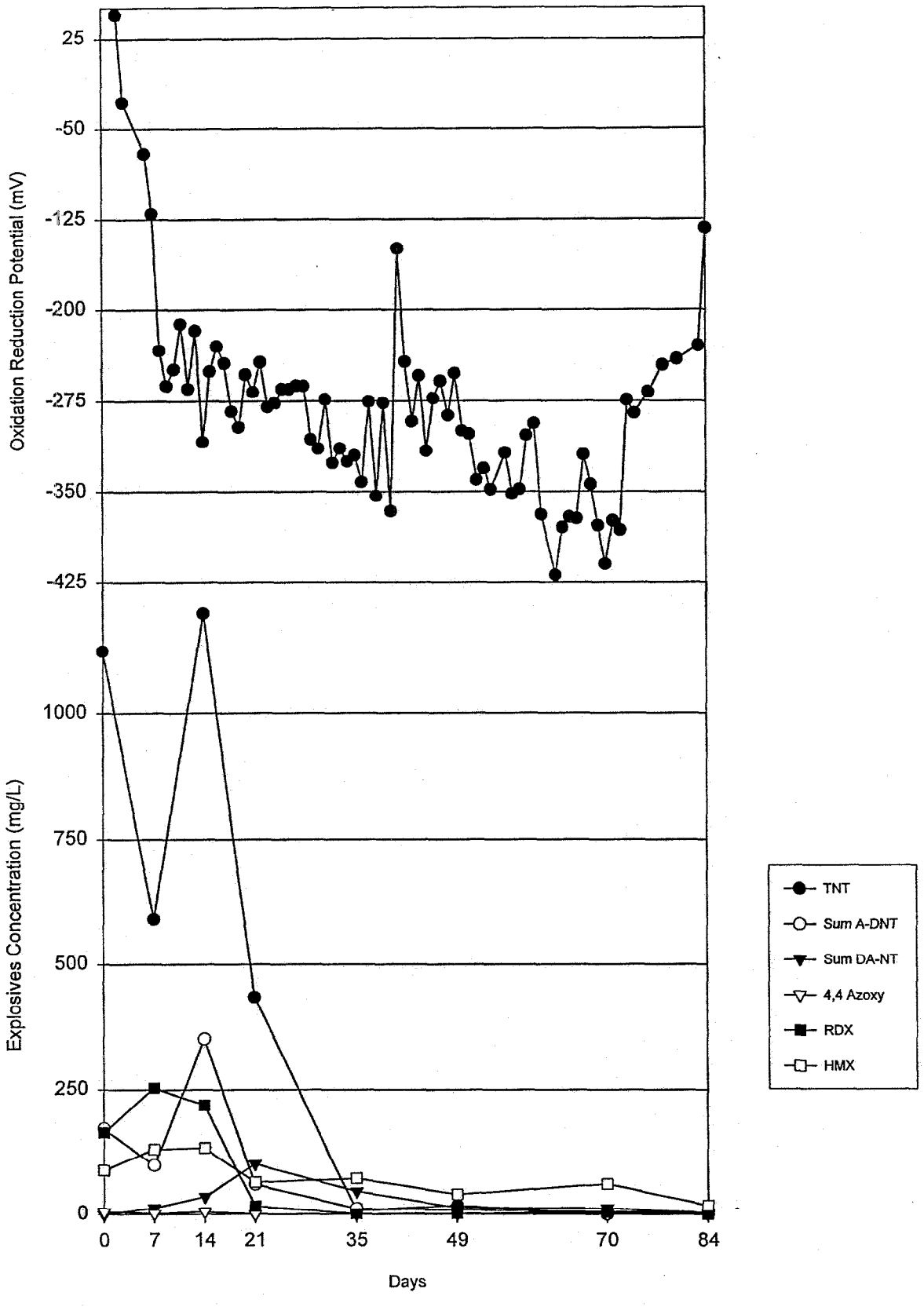


Figure 3-35 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic biocell Tween 80 and Molasses treatment

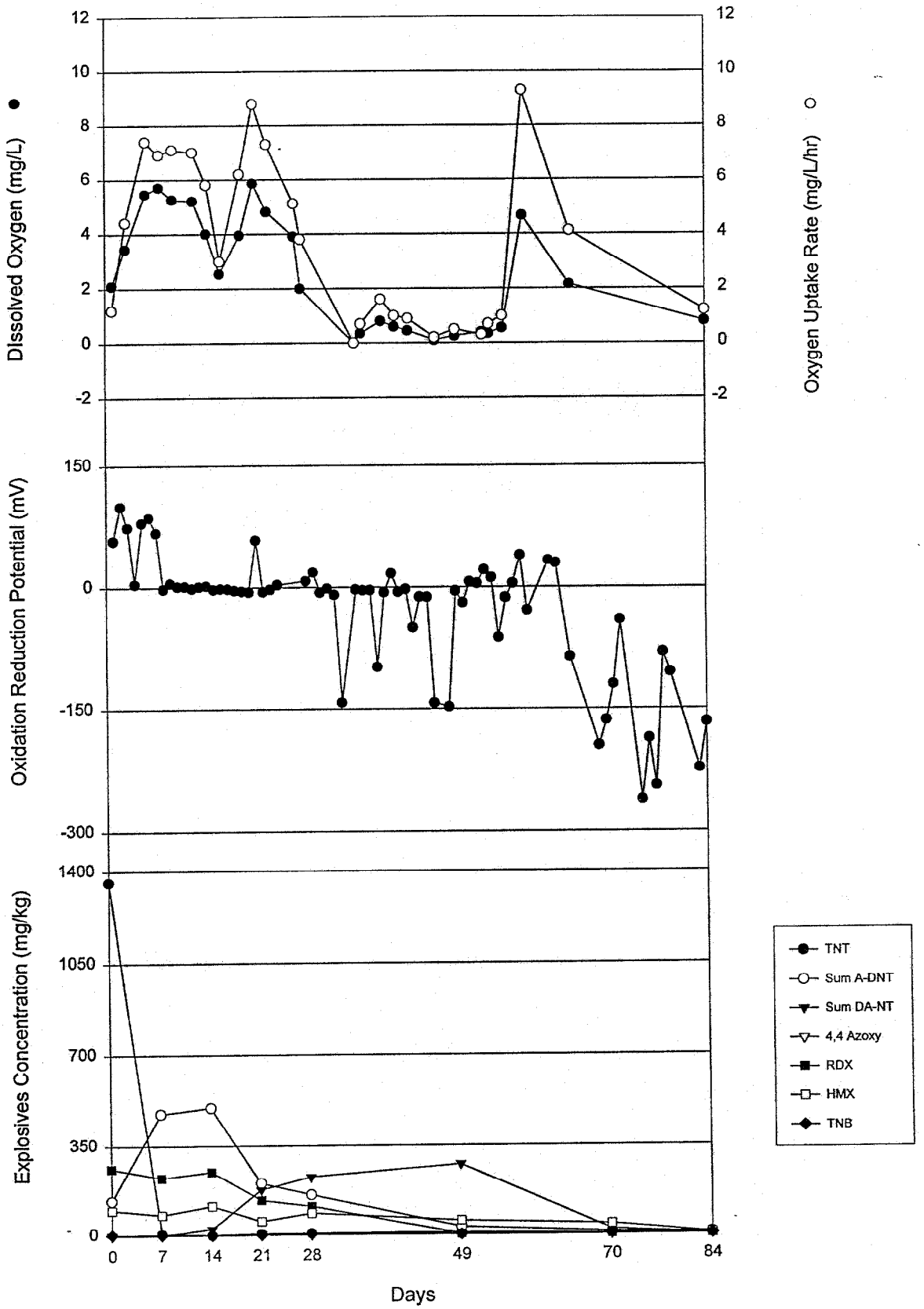


Figure 3-36 Plots of dissolved oxygen, oxygen uptake rate, oxidation reduction potential, and explosives concentration versus time for the aerated bioslurry Tween 80 and Molasses treatment

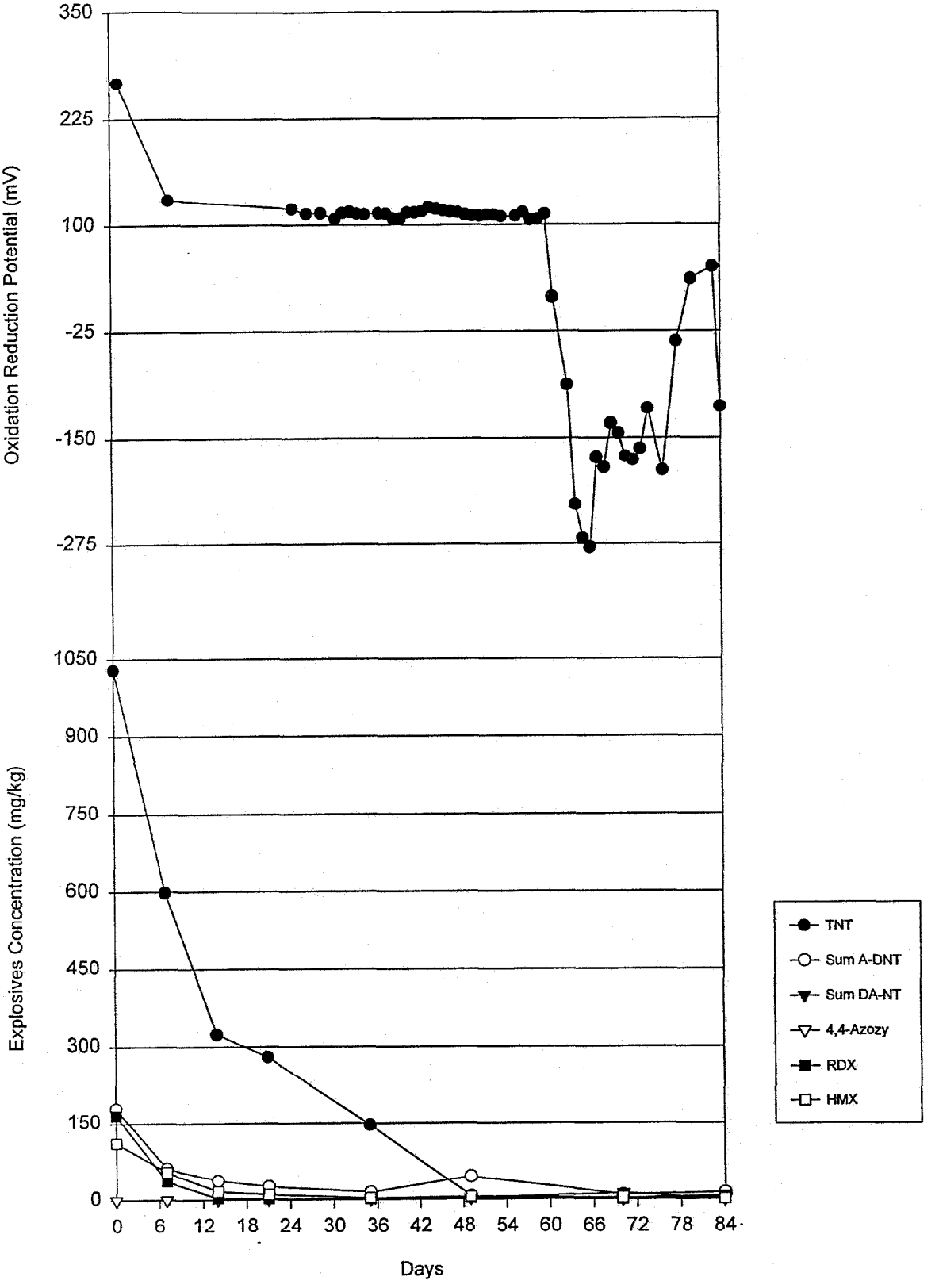


Figure 3-37 Plots of oxidation reduction potential and explosives concentration versus time for the anaerobic biocell Sterile Control treatment

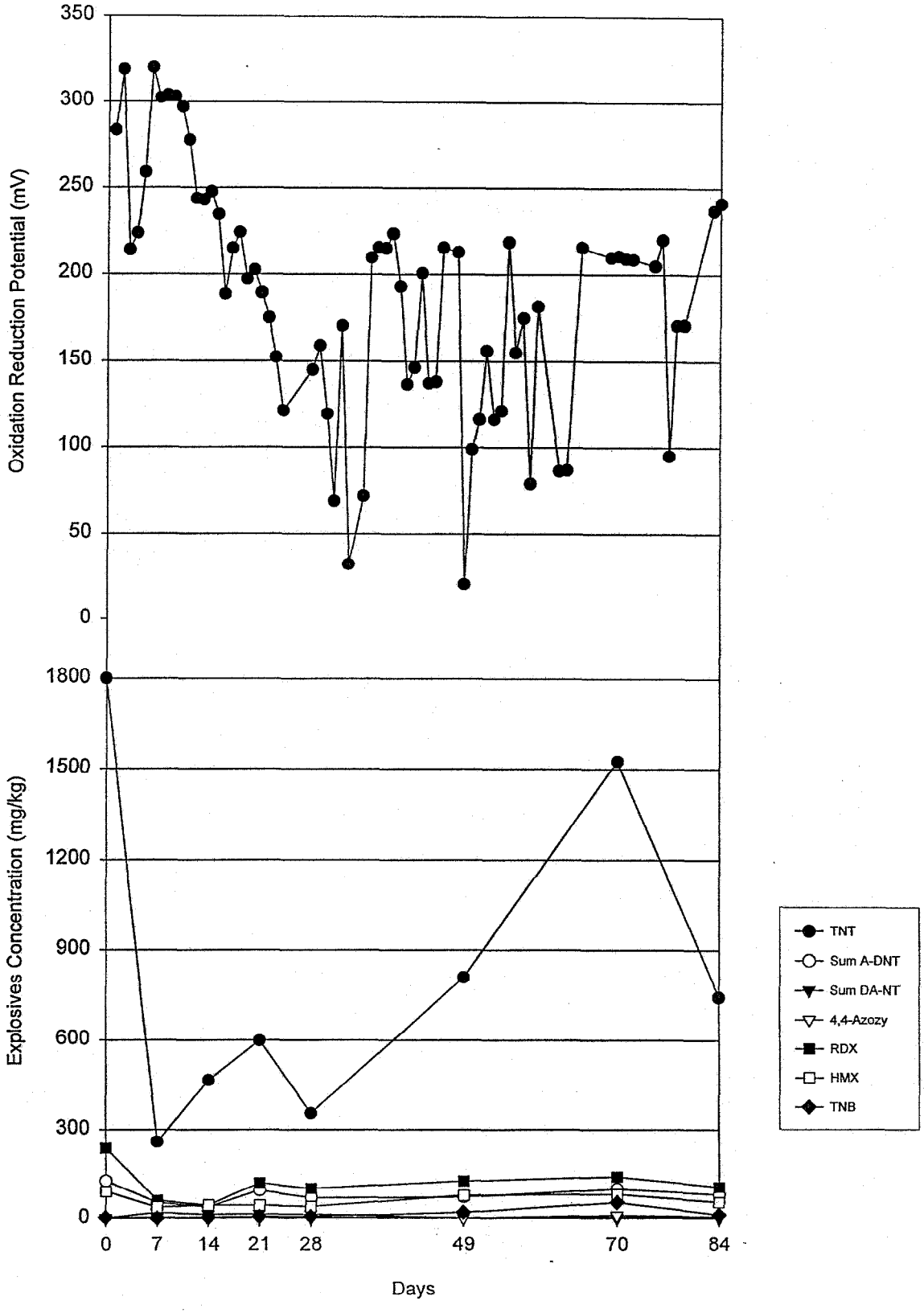


Figure 3-38 Plots of oxidation reduction potential and explosives concentration versus time for the non-aerated bioslurry Sterile Control treatment

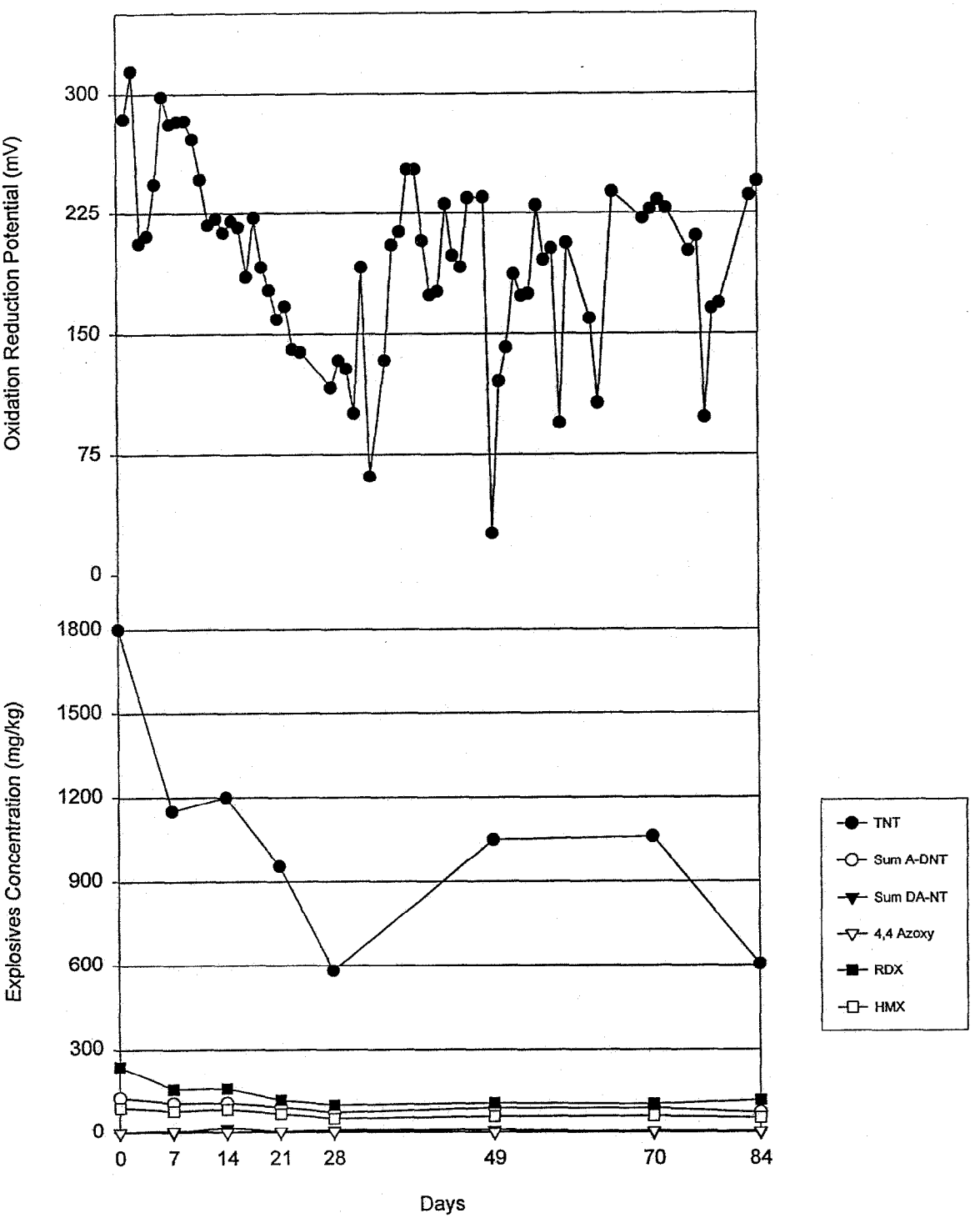


Figure 3-39 Plots of oxidation reduction potential and explosives concentration versus time for the aerated bioslurry Sterile Control treatment

Appendix A: Bioslurry Data

Table A-1. Bench-scale explosives data: non-aerated Sterile Control bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	2.61	34.10	0.71	58.40	4.36	3.50	< 1.00	< 2.00	< 0.500	
Day 28	2.71	30.40	1.07	56.60	4.24	3.33	< 1.00	< 2.00	0.00	
Day 49	3.04	33.50	2.34	72.70	3.98	3.23	<1.00	< 0.20	< 0.500	
Day 70	2.88	27.60	2.89	54.60	3.35	2.99	< 0.10	0.22	< 0.500	
Day 84	3.08	25.60	3.52	55.00	2.47	2.48	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	36.40	59.40	< 1.00	259.00	36.20	16.00	< 5.00	18.30	1.70	427.00
Day 14	43.80	44.50	1.60	464.00	23.00	15.20	< 5.00	12.70	0.270 J	605.07
Day 21	36.10	28.20	2.25	442.00	48.30	24.60	9.23	3.96 J	1.99	596.63
Day 28	31.60	19.30	2.50	203.00	30.50	17.60	10.10	3.20	1.05	318.85
Day 49	68.00	35.60	12.40	616.00	30.20	21.10	0.00	0.00	< 10.0	783.30
Day 70	72.00	66.50	45.00	1380.00	43.20	37.50	< 0.500	.202 J	8.08	1654.76
Day 84	44.20	35.70	< 0.100	598.00	39.20	26.00	< 0.100	< 0.100	3.16	746.26

Table A-2. Bench-scale explosives data: aerated Sterile Control bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	2.92	29.60	0.78	74.70	3.87	3.33	< 1.00	< 2.00	< 0.500	
Day 28	2.84	28.70	1.16	77.30	3.77	3.27	< 1.00	< 2.00	< 0.500	
Day 49	2.99	23.20	1.56	51.60	2.94	3.09	< 1.00	< 0.20	< 0.500	
Day 70	2.81	22.60	2.13	44.80	2.69	2.90	< 0.10	0.062 J	< 0.500	
Day 84	4.64	33.70	3.65	81.40	2.32	2.74	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	78.10 J	157.00	< 1.00	1150.00	66.00	40.00	< 5.00	< 10.0	5.61	1496.71
Day 14	84.40	159.00	2.65	1200.00	61.40	45.90	< 5.00	16.80	0.325 J	1570.83
Day 21	60.00	39.40	3.00	755.00	43.50	29.80	2.46 J	< 10.0	4.34	937.50
Day 28	43.10	22.80	2.48	375.00	33.30	20.70	6.18	2.50	2.38	508.44
Day 49	50.40	44.60	13.40	910.00	39.00	34.00	6.18	2.50	< 10.0	1100.08
Day 70	52.90	43.60	18.30	939.00	40.50	34.30	< 0.500	0.583 J	5.32	1135.38
Day 84	40.40	26.90	< 0.100	386.00	33.10	25.20	< 0.100	< 0.100	2.90	514.50

Table A-3. Bench-scale explosives data: anaerobic Simplot 4A bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.31	24.50	< 0.20	5.21	45.70	18.60	< 1.00	7.58	1.32
Day 28	2.44	25.80	< 0.20	< 0.20	20.70	14.90	< 1.00	61.80	0.098 J
Day 49	0.39	0.37	< 0.20	< 0.20	0.20	0.170 J	30.70	1.48 J	< 0.050
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	6.03	< 0.20	< 0.050
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	66.50	186.00	1.75	2680.00	53.80	33.60	< 5.00	< 10.0	3.50	3025.15
Day 7	131.00	337.00	3.15	472.00	197.00	94.70	< 5.00	< 10.0	112.00	1346.85
Day 14	116.00	356.00	3.65	302.00	456.00	203.00	3.50 J	3.35 J	21.50	1465.50
Day 21	88.60	174.00	0.750 J	171.00	354.00	172.00	1.73 J	< 10.0	156.00	1118.08
Day 28	42.90	78.90	0.29	11.90	419.00	152.00	< 0.500	16.90	332.00	1053.99
Day 49	5.15	2.30	< 1.00	19.20	21.20	17.80	0.00	0.00	< 10.0	65.65
Day 70	1.99	0.28	0.37	4.04	5.42	2.34	< 1.00	< 1.00	6.17	20.60
Day 84	2.10	0.29	< 0.100	4.08	3.94	2.68	< 0.500	.346 J	3.25	16.64

Table A-4. Bench-scale explosives data: anaerobic Simplot 4B bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.14	23.60	< 0.20	1.59	49.20	17.60	< 1.00	18.90	3.14
Day 28	2.39	23.60	< 0.20	0.94	69.10	23.90	< 1.00	10.10	1.95
Day 49	2.41	1.27	< 0.20	< 0.20	< 0.20	1.34	2.61	271.00	< 0.50
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	7.31	< 0.200	< 0.50
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	132.00	321.00	2.55	632.00	144.00	78.20	< 5.00	<10.0	46.10	1355.85
Day 14	109.00	323.00	4.85	475.00	340.00	169.00	4.84 J	18.00	14.70	1459.09
Day 21	126.00	214.00	0.350 J	40.80	338.00	146.00	< 5.00	<10.0	164.00	1029.15
Day 28	66.80	110.00	0.29	28.50	462.00	227.00	< 0.500	< 1.00	0.00	894.69
Day 49	90.4	9.85	1.3	80.4	51.2	32.2	0.00	0.00	< 10.0	265.35
Day 70	3.14	4.08	0.37	5.24	27.80	19.60	< 0.500	< 1.00	9.67	69.90
Day 84	1.86	0.21	< 0.100	3.16	4.26	2.24	< 0.500	.52 J	5.41	17.64

Table A-5. Bench-scale explosives data: anaerobic Simplot A bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.32	23.20	< 0.20	2.79	26.10	9.69	< 1.00	< 2.00	< 0.500
Day 28	2.55	24.40	< 0.20	3.55	27.10	10.50	< 1.00	< 2.00	0.249 J
Day 49	2.90	30.00	< 0.20	0.23	24.90	10.40	< 1.00	2.71	< 0.500
Day 70	2.94	27.70	< 0.020	< 0.020	17.90	2.91	< 0.100	1.94	0.399 J
Day 84	3.64	24.40	< 0.020	< 0.020	3.47	0.06	< 0.100	4.01	0.18

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	95.90	286.00	2.85	1380.00	96.20	64.90	< 5.00	< 10.0	2.48	1928.33
Day 7	114.00	292.00	3.90	558.00	147.00	79.90	< 5.00	< 10.0	92.20	1287.00
Day 14	106.00	346.00	7.25	470.00	411.00	206.00	3.37 J	< 10.0	13.20	1563.27
Day 21	79.80	169.00	< 1.00	141.00	275.00	130.00	< 5.00	< 10.0	465.00	1259.80
Day 28	112.00	199.00	0.50	41.60	234.00	111.00	0.27	4.52	326.00	1029.26
Day 49	48.40	108.00	1.40	42.00	284.00	153.00	0.00	0.00	116.00	752.80
Day 70	99.30	154.00	0.33	12.30	243.00	60.60	< 0.500	< 1.00	506.00	1075.64
Day 84	94.50	77.80	< 0.100	10.10	97.70	12.10	< 0.500	12.10	141.00	445.30

Table A-6. Bench-scale explosives data: anaerobic Simplot B bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.54	25.00	< 0.20	2.76	25.60	10.00	< 1.00	< 2.00	0.57
Day 28	2.53	24.60	< 0.20	5.77	25.10	10.50	< 1.00	< 2.00	0.194 J
Day 49	2.47	26.20	< 0.20	2.76	24.70	11.30	< 1.00	3.22	< 0.50
Day 70	2.51	22.20	< 0.020	0.04	22.00	8.28	1.34	1.99	0.187J
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	14.40	< 0.20	< 0.50

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	107.00	293.00	6.80	1080.00	175.00	106.00	< 5.00	< 10.0	100.00	1868.60
Day 14	120.00	358.00	8.80	841.00	333.00	198.00	1.81 J	8.77J	19.10	1889.08
Day 21	69.00	141.00	1.50	462.00	290.00	145.00	2.26 J	< 10.0	314.00	1424.76
Day 28	98.70	169.00	0.80	200.00	291.00	150.00	17.50	< 1.00	372.00	1299.28
Day 49	55.90	143.00	0.450 J	16.90	320.00	165.00	0.00	0.00	131.00	832.25
Day 70	72.80	144.00	0.56	12.90	257.00	116.00	< 0.50	< 1.00	0.00	603.26
Day 84	27.20	0.62	< 0.100	7.50	13.80	6.94	2.60	30.70	61.60	150.96

Table A-7. Bench-scale explosives data: anaerobic Potato Starch A bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.17	22.80	< 0.20	0.71	37.10	13.70	< 1.00	17.70	0.138 J
Day 28	2.28	22.90	< 0.20	1.58	34.20	< 0.20	< 1.00	14.60	.203 J
Day 49	2.58	23.70	< 0.20	< 0.20	30.00	9.86	< 1.00	22.10	< 0.50
Day 70	2.79	< 0.020	< 0.020	< 0.020	0.13	0.08	1.37	32.90	0.172J
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	82.30	205.00	2.25	2160.00	62.40	50.00	< 5.00	< 10.0	4.15	2566.10
Day 7	144.00	346.00	2.10	846.00	208.00	113.00	< 5.00	< 10.0	176.00	1835.60
Day 14	117.00	358.00	2.70	494.00	440.00	224.00	3.44J	3.68J	39.80	1683.07
Day 21	123.00	232.00	0.40 J	81.80	306.00	136.00	< 5.00	3.18 J	396.00	1278.38
Day 28	106.00	180.00	0.26	26.40	329.00	144.00	< 0.500	28.00	423.00	1236.66
Day 49	65.80	132.00	< 1.00	15.10	358.00	138.00	0.00	0.00	108.00	816.90
Day 70	62.20	3.10	< 0.100	8.78	39.40	9.79	< 0.50	< 1.00	331.00	455.24
Day 84	5.84	0.40	< 0.100	3.95	8.44	3.56	< 0.500	1.62	26.20	50.01

Table A-8. Bench-scale explosives data: anaerobic Potato Starch B bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY
Day 21	2.24	22.50	< 0.20	0.41	32.20	8.74	< 1.00	11.40	0.429 J
Day 28	2.53	20.80	< 0.20	1.84	15.10	< 0.20	< 1.00	11.60	0.123 J
Day 49	2.63	22.90	< 0.20	< 0.20	22.30	5.99	< 1.00	62.10	< 0.500
Day 70	2.16	0.04	< 0.020	< 0.020	< 0.020	0.04	0.33	0.37	< 0.500
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	135.00	317.00	2.40	1010.00	205.00	106.00	< 5.00	< 10.0	162.00	1938.00
Day 14	104.00	338.00	3.70	572.00	429.00	187.00	3.08J	2.80J	52.10	1692.03
Day 21	98.70	170.00	0.750 J	183.00	320.00	117.00	< 5.00	2.45 J	567.00	1458.90
Day 28	81.70	140.00	0.22	50.60	268.00	87.10	0.76	33.80	533.00	1195.45
Day 49	74.20	136.00	< 1.00	36.10	290.00	91.60	0.00	0.00	12.50	640.40
Day 70	51.60	1.08	0.43	7.11	19.80	6.51	< 0.500	< 1.00	212.00	0.00
Day 84	1.26	0.26	< 0.100	3.32	7.06	2.40	< 0.500	1.08	30.40	0.00

Table A-9. Bench-scale explosives data: aerated Tween 80 & Molasses A bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	3.20	23.30	< 0.20	< 0.20	26.70	4.06	59.20	18.00	< 0.500	
Day 28	3.74	15.40	< 0.20	< 0.20	6.31	3.56	20.80	40.50	0.070 J	
Day 49	3.24	< 0.20	< 0.20	< 0.20	6.44	< 0.20	1.93	84.20	< 0.500	
Day 70	2.73	< 0.020	< 0.020	< 0.020	0.17	< 0.020	1.90	0.59	< 0.500	
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	107.00	283.00	2.45	913.00	76.80	67.10	< 5.0	< 10.0	2.92	1452.27
Day 7	68.20	184.00	< 1.00	3.65	255.00	152.00	< 5.0	< 10.0	2.49	665.34
Day 14	130.00	224.00	< 1.00	< 1.00	254.00	113.00	1.47J	35.70	< 1.00	758.17
Day 21	34.80	62.20	< 1.00	3.20	49.40	13.60	<5.00	7.07J	4.67	174.94
Day 28	54.80	22.60	1.06	4.80	25.80	8.38	< 0.500	20.90	1.34	139.68
Day 49	49.40	1.50	< 1.00	8.05	6.70	5.90	0.00	0.00	< 10.0	71.55
Day 70	33.70	0.33	< 0.100	2.75	4.54	2.73	< 0.500	< 1.00	< 1.00	
Day 84	3.71	< 0.100	< 0.100	1.69	1.10	3.80	< 0.500	.579 J	< 1.00	

Table A-10. Bench-scale explosives data: aerated Tween 80 & Molasses B bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	3.61	28.40	< 0.20	< 0.20	40.40	6.22	5.73	40.80	< 0.500	
Day 28	4.08	25.90	< 0.20	< 0.20	31.10	3.45	46.20	43.30	0.130 J	
Day 49	4.79	< 0.20	< 0.20	< 0.20	5.13	< 0.20	14.40	106.00	< 0.500	
Day 70	4.15	0.21	< 0.020	< 0.020	0.45	< 0.020	2.02	9.00	< 0.500	
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	92.6 J	265.00	0.6 J	5.70 J	307.00	236.00	< 5.00	< 10.0	2.62	909.52
Day 14	98.60	271.00	< 1.00	1.05	422.00	207.00	3.69 J	6.68 J	< 1.00	1010.02
Day 21	55.10	73.50	< 1.00	6.60	112.00	24.90	< 5.00	15.80	2.49	290.39
Day 28	93.90	89.70	1.62	7.16	139.00	21.90	< 0.500	30.40	< 0.500	383.68
Day 49	36.20	2.30	< 1.00	7.25	7.20	7.55	0.00	0.00	< 10.0	60.50
Day 70	26.80	0.47	0.79	2.91	6.08	3.06	< 1.00	< 1.00	< 1.00	0.00
Day 84	5.28	< 0.100	< 0.100	1.78	5.11	3.52	.029 J	1.10	< 1.00	0.00

Table A-11. Bench-scale explosives data: aerated No Additives A bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	2.39	29.10	< 0.20	58.90	6.36	4.08	< 1.00	< 2.00	< 0.500	
Day 28	2.69	28.90	< 0.20	62.60	6.11	4.19	< 1.00	< 2.00	< 0.500	
Day 49	2.87	31.70	0.160 J	63.80	5.42	4.16	< 1.00	2.92	< 0.500	
Day 70	3.09	28.30	0.18	47.80	4.92	4.07	1.12	0.25	< 0.500	
Day 84	3.77	35.20	0.56	60.00	5.34	4.68	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	97.40	234.00	1.50	1870.00	70.80	53.20	< 5.00	< 10.0	4.15	2331.05
Day 7	125.00	339.00	2.50	1830.00	102.00	66.80	< 5.00	< 10.0	0.795 J	2466.10
Day 14	125.00	380.00	6.05	2130.00	124.00	86.70	< 5.00	< 10.0	0.840 J	2852.59
Day 21	92.30	187.00	5.80	1880.00	92.60	69.60	< 5.00	< 10.0	8.12	2335.42
Day 28	102.00	186.00	6.44	1760.00	79.20	61.50	0.45	< 1.00	9.76	2205.64
Day 49	70.00	143.00	9.50	1840.00	95.20	81.00	0.00	0.00	< 10.0	2238.70
Day 70	70.40	123.00	9.08	1660.00	68.60	63.10	12.80	< 1.00	< 1.00	2008.73
Day 84	84.60	111.00	< 0.100	1320.00	60.10	59.00	< 0.100	< 0.100	13.30	1648.00

Table A-12. Bench-scale explosives data: aerated No Additives B bioslurry

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	
Day 21	2.63	30.40	< 0.20	58.60	5.97	4.08	< 1.00	< 2.00	< 0.500	
Day 28	2.80	29.30	< 0.20	61.70	5.70	4.10	< 11.00	< 2.00	< 0.500	
Day 49	3.10	33.70	0.150 J	63.20	5.20	4.09	< 1.00	2.16	< 0.500	
Day 70	3.34	31.40	0.14	53.30	4.62	3.92	< 0.10	0.28	< 0.500	
Day 84	3.82	35.00	0.37	59.90	5.11	4.46	< 0.100	< 0.020	< 0.020	
Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	89.82	238.80	2.16	1800.60	72.00	53.76	0.00	0.00	0.00	2257.14
Day 7	131.00	342.00	2.30	1860.00	88.60	64.10	< 5.00	< 10.0	5.22	2493.22
Day 14	117.00	326.00	5.00	2350.00	109.00	81.30	< 5.00	< 10.0	0.650 J	2989.65
Day 21	100.00	190.00	5.40	2020.00	91.00	79.40	< 5.00	< 10.0	4.18	2489.98
Day 28	116.00	178.00	5.49	1910.00	90.00	72.70	1.46	< 1.00	7.94	2381.88
Day 49	94.80	128.00	12.50	1490.00	76.80	76.60	0.00	0.00	< 10.0	1878.70
Day 70	77.30	124.00	9.26	1720.00	68.50	64.40	< 0.100	< 0.100	9.93	2075.40
Day 84	94.70	104.00	< 0.100	1270.00	61.10	59.40	< 0.100	< 0.100	8.39	1597.59

Appendix B: Biocell Data

Table B-1. Bench-scale explosives data: anaerobic Tween & Molasses A biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	0.60 J	24.6	< 2.00	< 2.00	< 2.00	1.50 J	< 10.0	< 100.0	< 0.500	26.70
Day 14	5.70	16.7	< 2.00	< 2.00	< 2.00	3.50	27.4	1.52 J	< 0.500	54.82
Day 21	7.60	0.500 J	< 2.00	< 2.00	2.10	2.50	15.20	49.00	< 0.500	76.90
Day 35	6.70	< 0.20	< 0.20	< 0.20	< 0.20	1.12	5.62	31.10	< 0.500	44.54
Day 49	0.89	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 1.00	9.16	< 0.500	10.05
Day 70	4.80	0.31	< 0.020	< 0.020	< 0.020	0.19	6.86	< 2.00	< 0.500	12.16
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	69.70	166.00	1.60 J	1220.00	104.00	63.40	< 5.00	< 10.0	11.70	1663.10
Day 7	123.00	254.00	2.20 J	970.00	76.00	45.80	< 5.00	< 10.0	2.68	1528.50
Day 14	130.00	328.00	< 2.50	2170.00	397.00	182.00	< 5.00	11.90	8.48	3304.28
Day 21	69.80	< 10.0	< 2.50	792.00	32.80	29.50	26.50	7.36	0.875 J	958.84
Day 35	61.20	1.25	< 1.00	4.00	5.15	4.80	3.83 J	< 10.00	< 10.00	80.23
Day 49	39.40	2.00	0.30 J	6.40	6.65	7.95	2.55 J	< 10.00	0.170 J	65.42
Day 70	83.10	7.33	< 0.100	< 0.100	3.68	4.15	4.24	0.581 J	< 1.00	103.08
Day 84	14.30	0.23	0.055 J	0.13	1.31	0.82	2.49	0.80	< 10.00	20.13

Table B-2. Bench-scale explosives data: anaerobic Tween 80 & Molasses B biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	1.52	22.60	< 0.20	< 0.20	0.21	2.41	6.88	7.52	< 0.500	41.14
Day 14	2.14	1.63	< 0.20	< 0.20	< 0.20	1.34	0.27J	7.22	< 0.500	12.60
Day 21	10.10	16.70	< 0.20	< 0.20	1.91	2.20	14.60	49.40	< 0.500	94.91
Day 35	7.12	< 0.20	< 0.20	< 0.20	1.55	< 0.20	3.50	25.50	< 0.500	37.67
Day 49	6.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	7.55	< 2.00	< 0.500	13.75
Day 70	5.74	0.13	< 0.020	< 0.020	< 0.020	0.16	4.71	< 2.00	< 0.500	10.73
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	111.36	165.20	0.70	1028.00	109.80	68.80	0.00	0.00	< 10.0	1483.86
Day 7	138.00	194.00	0.70 J	211.00	41.80	31.60	< 5.00	8.42 J	1.05 J	626.57
Day 14	129.00	88.00	0.25 J	226.00	70.10	47.10	< 5.00	14.40	2.20 J	577.25
Day 21	39.50	11.65	0.20 J	78.00	27.80	20.90	< 5.00	10.80	< 10.00	189.10
Day 35	66.90	1.00	< 1.00	9.40	3.95	4.70	3.27J	3.20 J	< 10.00	92.67
Day 49	32.20	0.500 J	< 1.00	29.80	1.70	2.25	2.66 J	< 10.0	0.720 J	69.83
Day 70	26.60	0.42	< 0.100	< 0.100	1.46	1.56	4.32	0.513 J	< 1.00	34.87
Day 84	20.60	0.35	< 0.100	1.01	2.03	2.98	3.08	< 1.00	< 10.00	30.05

Table B-3. Bench-scale explosives data: anaerobic Molasses A biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	0.700 J	16.4	< 0.20	< 0.20	12.60	1.20	< 0.20	12.30 J	< 0.500	43.20
Day 14	2.80	2.00	< 0.20	< 0.20	< 0.20	0.90 J	< 0.20	< 0.20	< 0.500	5.70
Day 21	< 0.20	< 0.20	< 0.20	< 0.20	0.400 J	< 0.20	13.60	22.10	< 0.500	36.10
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	50.20	7.84	< 0.500	58.04
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	14.80	< 2.00	< 0.500	14.80
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 1.00	0.112 J	< 0.500	0.11
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.10	< 0.20	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	68.90	186.00	< 2.50	1110.00	200.00	106.00	< 5.00	< 10.0	35.50	1670.90
Day 7	51.00	146.00	< 2.50	1460.00	85.20	70.00	< 5.00	< 10.0	2.62	1812.20
Day 14	120.00	92.80	< 2.50	444.00	45.70	46.10	< 5.00	8.03	< 10.00	756.63
Day 21	21.20	13.80	< 2.50	218.00	78.20	68.70	8.71	13.90	5.91	422.51
Day 35	32.00	7.35	0.65 J	200.00	47.40	40.00	14.70	4.45 J	< 10.00	346.95
Day 49	8.75	< 1.00	< 1.00	247.00	6.20	3.45	4.31 J	< 10.0	1.32	269.71
Day 70	0.86	13.50	< 0.100	0.21	3.40	1.98	0.145 J	0.800 J	< 10.00	20.90
Day 84	11.50	0.47	0.13	99.60	4.42	4.72	2.91	0.711 J	2.39	126.85

Table B-4. Bench-scale explosives data: anaerobic Molasses B biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	1.07	13.90	< 0.20	< 0.20	10.80	2.05	<1.00	90.00	< 0.500	117.82
Day 14	7.03	22.10	< 0.20	< 0.20	< 0.20	5.12	< 1.00	14.60	< 0.500	48.85
Day 21	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	51.10	61.40	< 0.500	112.50
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	43.90	2.62	< 0.500	46.52
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	16.30	< 2.00	< 0.500	16.30
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	0.188 J	< 0.500	0.19
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	111.36	165.20	0.70	1028.00	109.80	68.80	0.00	0.00	0.00	1483.86
Day 7	47.00	128.00	0.60 J	1275.00	172.00	88.10	< 5.00	4.44 J	4.10 J	1719.99
Day 14	96.00	91.20	1.75	1350.00	246.00	110.00	< 5.00	41.00	4.20 J	1940.85
Day 21	29.50	8.15	0.250 J	354.00	50.00	38.60	6.72	8.89 J	1.10 J	497.46
Day 35	4.65	0.60 J	0.30 J	107.00	8.80	7.80	11.30	< 10.0	< 10.00	140.70
Day 49	4.05	6.45	< 1.00	10.00	17.90	15.20	3.07 J	< 10.0	1.07	57.74
Day 70	2.14	7.33	< 0.100	0.46	9.54	9.06	0.83	1.22	< 1.00	30.58
Day 84	1.59	0.65	< 0.100	7.24	2.10	1.58	0.309 J	0.597 J	1.30	15.36

Table B-5. Bench-scale explosives data: anaerobic Simplot A biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	2.80	30.00	< 2.00	42.70	21.30	9.80	< 10.0	< 20.0	< 0.500	106.60
Day 14	2.10	24.6	< 2.00	< 2.00	25.10	3.20	4.42 J	43.70	0.333 J	103.45
Day 21	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	6.30 J	64.10	< 0.500	70.40
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	31.60	12.60	< 0.500	44.20
Day 49	< 0.20	< 0.20	< 0.20	< 2.00	< 2.00	< 2.00	2.17	3.67	< 0.500	5.84
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	1.41	< 2.00	< 0.500	1.41
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	276.00	119.00	< 2.50	790.00	73.60	44.50	< 5.00	< 10.0	2.05	1305.15
Day 7	81.70	202.00	< 2.50	742.00	80.10	69.70	< 5.00	< 10.0	3.50	1179.00
Day 14	94.00	256.00	5.85	1140.00	182.00	91.80	<5.00	< 10.0	2.08	1771.73
Day 21	12.20	4.05 J	0.55 J	515.00	16.80	14.20	7.16	36.60	0.900 J	607.46
Day 35	9.30	3.25	0.25 J	13.7	20.8	15.0	9.74	1.06 J	< 10.00	73.10
Day 49	7.50	2.45	< 1.00	154	13.8	7.30	6.30	< 10.00	0.970 J	192.32
Day 70	33.5	4.25	0.60	4.96	25.2	28.1	5.48	6.92	< 1.00	109.43
Day 84	7.36	1.12	< 0.100	0.58	5.16	5.82	0.517	0.376 J	< 10.00	21.09

Table B-6. Bench-scale explosives data: anaerobic Simplot B biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	2.34	23.40	0.070 J	36.50	21.60	9.11	< 1.00	7.90	0.337 J	101.26
Day 14	2.29	17.10	< 0.20	< 0.20	7.10	3.98	4.54	262.00	< 0.500	297.01
Day 21	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	53.00	269.00	< 0.500	322.00
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	30.00	8.55	< 0.500	38.55
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 1.00	< 2.00	< 0.500	0.00
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	0.571 J	0.132J	< 0.500	0.70
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	111.36	165.20	0.70	1028.00	109.80	68.80	0.00	0.00	< 10.0	1483.86
Day 7	64.90	128.00	0.70 J	1084.00	83.40	46.80	< 5.00	< 10.0	< 10.0	1408.40
Day 14	149.00	337.00	< 1.00	112.00	467.00	170.00	< 5.00	34.40	< 10.0	1269.60
Day 21	20.20	4.65	0.60 J	217.00	22.00	19.40	11.00	42.60	< 10.0	337.45
Day 35	17.30	2.45	0.40 J	284.00	24.60	25.80	8.31	3.92 J	< 10.0	367.18
Day 49	3.90	2.90	< 1.00	18.60	14.00	13.60	3.20	< 10.0	0.295 J	56.50
Day 70	2.28	1.22	0.18	< 0.100	7.42	8.52	0.58	0.976 J	< 1.00	21.24
Day 84	3.71	< 0.100	1.10	118.00	2.10	1.70	< 0.500	0.949 J	< 10.0	127.56

Table B-7. Bench-scale explosives data: anaerobic Potato Starch A biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	2.40	26.10	< 2.00	37.40	19.80	8.90	< 10.0	< 20.0	0.307 J	94.91
Day 14	2.40	22.8	< 2.00	< 2.00	6.60	2.80	< 10.0	33.00	0.194 J	67.79
Day 21	1.30 J	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	1.54 J	66.60	< 0.500	69.44
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	36.70	12.60	< 0.500	49.30
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	15.10	3.62	< 0.500	18.72
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	0.471 J	0.278 J	< 0.500	0.75
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	51.50	144.00	0.800 J	920.00	92.20	59.00	< 5.00	< 10.0	1.86	1269.36
Day 7	90.60	102.00	0.60 J	187.00	202.00	69.70	< 5.00	67.90	28.60	748.40
Day 14	72.00	186.00	< 2.50	2040.00	98.20	48.70	< 5.00	< 10.0	2.19	2447.09
Day 21	41.30	4.35 J	< 2.50	55.60	18.40	11.40	7.47	70.80	2.30	211.62
Day 35	9.70	< 1.00	0.30 J	150.00	12.40	9.95	11.10	2.41 J	< 10.00	196.01
Day 49	3.45	< 1.00	0.10 J	36.20	13.60	13.60	6.18	< 10.0	0.905 J	74.04
Day 70	2.34	1.44	0.40	8.94	11.80	10.90	2.73	0.876 J	< 1.00	39.49
Day 84	1.62	0.23	< 0.100	2.34	1.26	0.79	< 0.500	0.394 J	< 10.00	6.66

Table B-8. Bench-scale explosives data: anaerobic Potato Starch B biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	1.88	23.20	< 0.20	30.10	17.60	8.35	< 1.00	6.36	0.186 J	87.68
Day 14	2.07	20.90	< 0.20	< 0.20	17.60	2.82	< 1.00	120.00	< 0.500	163.39
Day 21	1.96	< 0.20	< 0.20	< 0.20	0.47	< 0.20	18.60	391.00	< 0.500	412.03
Day 35	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	25.20	12.00	< 0.500	37.20
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	13.90	< 2.00	< 0.500	13.90
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	1.10	0.162 J	< 0.500	1.26
Day 84	< 0.20	< 0.20	< 0.20	< 0.020	< 0.020	< 0.020	< 0.100	< 0.200	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	111.36	165.20	0.70	1028.00	109.80	68.80	< 1.00	< 1.00	< 10.0	1483.86
Day 7	62.80	198.00	1.30	2340.00	96.40	53.70	< 5.00	< 10.0	< 10.0	2752.20
Day 14	142.00	307.00	0.45 J	268.00	300.00	128.00	< 5.00	4.62 J	< 10.0	1150.42
Day 21	100.00	18.40	0.50 J	2000.00	62.20	37.70	108.00	21.70	< 10.0	2349.40
Day 35	12.4	2.4	0.40 J	27.6	17.6	12.00	10.40	< 10.0	< 10.0	82.95
Day 49	8.5	< 1.00	< 1.00	234	8.00	5.90	6.82	3.81 J	0.570 J	267.60
Day 70	5.26	2.02	0.71	15.2	17.3	17.6	2.01	2.41	< 1.00	62.63
Day 84	1.89	1.62	0.23	13.6	5.86	3.86	0.362 J	0.477 J	1.63	29.59

Table B-9. Bench-scale explosives data: non-aerated Sterile Control biocell

Water Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	SUM
Day 7	0.45	2.46	< 0.20	2.78	0.44	0.32	< 1.00	< 2.00	< 0.500	6.45
Day 14	< 0.20	< 0.20	< 0.20	0.050 J	< 0.20	< 0.20	< 1.00	< 2.00	< 0.500	0.05
Day 21	1.96	< 0.20	< 0.20	< 0.20	0.47	< 0.20	< 1.00	< 2.00	< 0.500	2.43
Day 35	< 0.20	< 0.20	< 0.20	0.080 J	< 0.20	< 0.20	< 1.00	< 0.20	< 0.500	0.08
Day 49	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 1.00	2.36	< 0.500	2.36
Day 70	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 1.00	4.71	< 0.500	4.71
Day 84	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.020	< 0.100	< 2.00	< 0.500	0.00

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL EXP
Initial	111.36	165.20	0.70	1028.00	109.80	68.80	< 1.00	< 1.00	< 10.00	1483.86
Day 7	54.30	33.10	0.100 J	595.00	34.80	26.20	< 5.00	< 10.0	0.470 J	743.97
Day 14	16.20	2.65	0.30 J	323.00	20.80	16.70	< 5.00	< 10.0	0.475 J	380.13
Day 21	7.30	1.50	0.40	280.20	13.20	12.00	< 1.00	< 1.00	< 1.00	314.60
Day 35	3.15	1.05	0.20 J	146.00	8.95	5.75	< 5.00	2.21 J	< 10.00	167.31
Day 49	5.30	2.65	0.750 J	6.05	24.90	20.40	< 5.00	< 10.0	1.59	61.64
Day 70	3.45	< 0.100	< 0.100	2.06	4.56	4.36	< 0.500	6.15	< 1.00	20.62
Day 84	0.79	4.89	< 0.100	7.74	6.89	5.88	< 5.00	0.437 J	< 10.00	26.63

Table B-10. Bench-scale explosives data: aerated Sterile Control biocell

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL
Initial	76.2	154	0.450 J	452.00	86.8	79.8	< 5.00	< 10.00	< 10.00	0.08
Day 7	51.4	112	< 1.00	2680.00	31.6	32.6	< 5.00	< 10.00	0.600 J	2.36
Day 14	73.3	119	0.350 J	612.00	35.6	40.4	< 5.00	< 10.00	0.595 J	4.71
Day 21	65.3	115	0.62	1105.00	48.3	45.1	< 1.00	< 1.00	< 1.00	0.00
Day 35	58.30	113.00	1.00	961.00	68.90	53.00	< 5.00	< 10.00	< 10.00	0.00
Day 49	54.00	94.00	0.750 J	1410.00	27.80	25.10	< 5.0	< 10.00	4.26	0.00
Day 70	51.60	107.00	1.28	1580.00	19.60	30.90	< 0.500	1.02	5.06	0.00
Day 84	70.40	94.80	0.58	1150.00	18.40	26.20	< 0.500	< 10.00	< 10.00	EXP

Table B-11. Bench-scale explosives data: aerated Tween 80 & Molasses A biocell

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL
Initial	90.70	211.00	1.10 J	1100.00	79.20	71.10	< 5.00	< 10.0	2.56	1555.66
Day 7	3680	407.00	< 2.50	1500.00	545.00	236.00	< 5.00	< 10.0	15.50	6383.50
Day 14	108.00	269.00	< 2.50	1540.00	160.00	83.40	< 5.00	< 10.0	2.63	2163.03
Day 21	79.10	199.00	< 2.50	1280.00	110.00	79.80	< 5.00	< 10.0	4.58	1752.48
Day 35	78.0	198	0.450 J	300	96.6	76.6	< 5.00	2.12 J	< 10.00	751.77
Day 49	69.2	173	< 1.00	759	93.0	59.3	< 5.00	< 10.0	4.00	1157.50
Day 70	65.6	140	0.25	327	93.6	52.2	< 5.00	0.664 J	< 1.00	679.78
Day 84	71.6	160	< 1.00	656	98.4	49.0	< 5.00	3.58 J	< 10.00	1038.58

Table B-12. Bench-scale explosives data: aerated Tween 80 & Molasses biocell

Soil Sample	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,6-DANT	2,4-DANT	4,4 AZOZY	TOTAL
Initial	111.36	165.2	0.7	1028	109.8	68.8	< 1.00	< 5.00	< 10.00	1483.86
Day 7	65.90	189.00	4.20	848.00	73.40	65.20	< 5.00	< 5.00	< 10.00	1246.10
Day 14	58.40	134.00	0.650 J	784.00	75.40	68.40	< 5.00	< 5.00	< 10.00	1121.35
Day 21	63.00	145.00	0.85 J	1000.00	85.90	74.80	< 5.00	< 5.00	< 10.00	1370.45
Day 35	59.60	140.00	0.20 J	493.00	93.00	58.60	< 5.00	3.17 J	< 10.00	847.72
Day 49	55.00	117.00	< 1.00	492.00	79.60	46.00	< 5.00	< 5.00	3.72	793.32
Day 70	62.30	135.00	0.89	275.00	79.90	42.00	< 0.500	4.20	1.28	601.19
Day 84	68.20	106.00	0.55	625.00	68.00	35.80	< 5.00	3.21 J	< 10.00	906.76

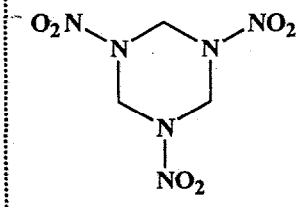
Appendix C:
Physical, chemical, and environmental data

Physical, chemical, and environmental data for representative explosives and explosives-associated compounds (XACs) are presented in a series of tables as follows (McGrath 1996):

Table C.1: RDX
Table C.2: HMX
Table C.3: 2,4,6-TNT
Table C.4: 1,3,5-TNB

Table C.1: Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) physical, chemical and environmental properties.

CAS No.: 121-82-4
Other names: hexogen; cyclotrimethylenetrinitramine; cyclonite; RDX ≡ Royal Demolition eXplosives (or Research & Development eXplosives)
Formula (semistrucural and empirical): $(NO_2)_3N_3C_3H_6$ or $C_3H_6N_6O_6$
Molecular Mass: 222.26 $g \cdot mol^{-1}$



Parameter	Value / Comments	Reference
Density	1.82 $g \cdot cm^{-3}$	Kaye (1980)
Melting Point	204-206 °C	Meyer (1977); Banerjee *et al.* (1980); Merck (1983)
Crystallography	orthorhombic crystals (from acetone); colorless	Verschueren (1983); Meyer (1977)
Dipole moment	Low (≈ 0); molecule is nearly symmetrical (out of plane configurations have non-zero dipole moment)	
Vapor Properties (RDX)		
Henry's Law Constant, K_H	1.96 E-11 atm·m^3·mol^{-1} (25°C; est.) 2 E-05 torr·L·mol^{-1} (°C; est.)	Rosenblatt *et al.* (1989) Spanggord *et al.* (1980)
Vapor Pressure	4.03 E-09 torr (25°C; est.)	Rosenblatt *et al.* (1980)
Aqueous Solubility (RDX)		
10° C	28.9 ± 1.0 $mg \cdot L^{-1}$	Sikka *et al.* (1980)
20° C	42.3 ± 0.6 $mg \cdot L^{-1}$	Sikka *et al.* (1980)
25° C (?)	45 $mg \cdot L^{-1}$	Spalding & Fulton (1988)
25 ± 0.2° C	59.9 ± 1.2 $mg \cdot L^{-1}$; 269 µM	Banerjee *et al.* (1980)
26.5°C	59.9 ± 1.4 $mg \cdot L^{-1}$	Sikka *et al.* (1980)
30° C	75.7 ± 1.1 $mg \cdot L^{-1}$	Sikka *et al.* (1980)
EPA Drinking Water Std. (DWEL)	10 $mg \cdot L^{-1}$	(from Rosenblatt *et al.*, 1980)
RMCL Recc.Max.Contam.Lev.	35 $\mu g \cdot L^{-1}$	DOA (1980; from Spalding & Fulton, 1988)
Solubility in Organic Solvents (RDX)		
Acetone	1 g / 25 mL (40 000 $mg \cdot L^{-1}$) 4.18 g / 100 g @ 0°C 8.38 g / 100 g @ 30°C	Merck (1983); Urbanski *et al.* (1983)
Benzene	0.055 g / 100 g @ 25°C 0.085 g / 100 g @ 30°C	Urbanski *et al.* (1983)
Toluene	0.016 g / 100 g @ 0°C 0.025 g / 100 g @ 25°C 0.050 g / 100 g @ 30°C	Urbanski *et al.* (1983)
Methanol	0.140 g / 100 g @ 0°C 0.325 g / 100 g @ 30°C	Urbanski *et al.* (1983)
Ethanol	0.040 g / 100 g @ 0°C 0.155 g / 100 g @ 30°C	Urbanski *et al.* (1983)
Acetic Acid (glacial)	slight	Merck (1983)

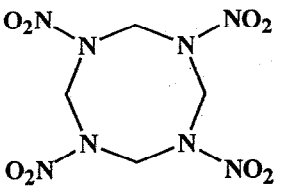
(continued)

Table C.1: RDX (continued)

Parameter	Value / Comments	Reference
Environmental Fate & Transport (RDX)		
Partitioning Coefficients	log k_{ow}: 0.87 ± 0.028 0.81 0.86	Banerjee *et al.* (1985) Major (1984) Jenkins (1989)
	log k_{oc}: 2.00 2.13 0.89, 1.87, and 2.43 1.62 and 2.10	Rosenblatt (1986; CAAP) Tucker et al. (1985) Sikka et al. (1980) Spanggord et al. (1980b)
	k_d: 0.2, 1.8, 6.4, and 7.8 0.8, 3.06, and 4.15 1.4 and 4.2 1.6 (nondimensional; kg-water / kg-soil); CAAP aquifer material with very low f_{oc}	Hale, Stanford, and Taft (1979) Sikka et al. (1980) Spanggord et al. (1980b) Tsai *et al.* (1985)
Diffusion	Water: 7.15 E-06 $cm^2 \cdot s^{-1}$ Air: 0.074 $cm^2 \cdot s^{-1}$	Rosenblatt *et al.* (1989)
Biodegradability	Aerobic: negligible	McCormick *et al.* (1981)
	Anaerobic: significant cometabolism Transformation products: methanol, hydrazine, formaldehyde, dimethylhydrazine (1,1-, 1,2-) which are mutagens	McCormick *et al.* (1981)
Toxicity	Possible carcinogen (USEPA); not mutagen; transformation products may be toxic	McCormick *et al.* (1981)
Photosensitivity	Rapid; not enhanced by humics substrate (sensitizer) Transformation products: nitrite, nitrate, formaldehyde, N_2, triazine (?)	Sikka *et al.* (1980) McCormick *et al.* (1981)
Hydrolysis	Insignificant	Rosenblatt *et al.* (1989)
Other Abiotic Reactions		
Aqueous Speciation	Not likely	—
Aqueous Complexation	No reports; probable, but weak	—
Abiotic Reduction	No reports; perhaps under anaerobic systems; probably not under aerobic conditions	—
Polymerization	No reports; perhaps in reduced (amino compounds) transformation products	—
Binding to Soil Solids	No reports; perhaps amino compounds	—

Table C.2: octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) physical, chemical and environmental properties.

CAS No.: 2691-41-0
Other names: Octagen; cyclo.tetramethylene.tetranitramine
Formula (semistrucural and empirical): $C_4H_8N_4(NO_2)_4$ or $C_4H_8N_8O_8$
Molecular Mass: 296.2 g mol^{-1}



Parameter	Value / Comment	Reference
Density	1.90 g cm^{-3} (β form)	Rosenblatt *et al.* (1989)
Melting Point	286° C	Rosenblatt *et al.* (1989)
Crystallography	Colorless crystals	Meyer (1977)
Dipole moment	Low (≈ 0); molecule is nearly symmetrical; non-zero DM may arise from out of plane configurations	—
Vapor Properties (HMX)		
Henry's Law Constant, K_H	2.60 E-15 atm m^3 mol^{-1} (25° C; est.)	Rosenblatt *et al.* (1989)
Vapor Pressure	25° C 3.33 E-14 torr 100° C 3. E-09 torr	Rosenblatt *et al.* (1989) Tucker et al (1985)
Aqueous Solubility (HMX) Leggett *et al.* (1977)		
10°C	1.21 ± 0.04 mg·L^{-1}, 4.09 µM	Spanggord *et al.* (1982b)
20° C	2.6 ± 0.01 mg·L^{-1}, 8.78 µM	Spanggord *et al.* (1982b)
22-25°C	5 mg·L^{-1} 16.8 µM	Glover & Hoffsommer (1973)
30°C	5.7 ± 0.1 mg·L^{-1}, 19.2 µM	Spanggord *et al.* (1982)
Solubility in Organic Solvents (HMX)		
Acetone	n.d.	—
Benzene	n.d.	—
Ethanol	n.d.	—
Acetic Acid (glacial)	n.d.	—
Environmental Fate & Transport (HMX)		
Partition Coefficients	log K_{ow}: 0.26 0.06	Major (1989) Jenkins (1989)
	log K_{oc}: 0.54 (est.) log K_{oc}: 2.83; for Holston River sediment (f_{oc}=0.013) based on measured k_p = 8.7	Rosenblatt *et al.* (1989); Spanggord *et al.* (1982)
	K_d: n.d.	—
	K_B: 63 (measured biosorption partitioning)	Spanggord *et al.* (1982)

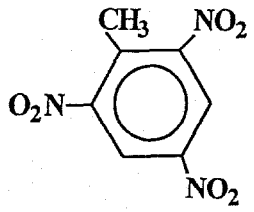
(continued)

Table C.2: HMX (continued)

Parameter	Value / Comment	Reference
Environmental Fate & Transport (HMX) — Continued		
Volatilization	Minor; k_v = 2.4 E-04 to 7.2 E-04 day^{-1} (1st-order rate constant); $t_{1/2}$ = 3000 to 1000 days (est. from experiments)	Spanggord *et al.* (1982b)
Diffusion	Water: 6.02 E-06 $cm^2 \cdot s^{-1}$ (est.) Air: 0.063 $cm^2 \cdot s^{-1}$ (est.)	Rosenblatt *et al.* (1989)
Biodegradation	Aerobic: negligible	Spanggord *et al.* (1982b)
	Anaerobic: slow; accelerated, 1° kinetics in the presence of primary substrate (cometabolism)	Spanggord *et al.* (1982b)
Toxicity	not carcinogenic	[in Rosenblatt *et al.*, 1989]
Photolysis	Significant; 1st-order k = 0.15 d^{-1} ($t_{1/2}$ = 5 d); for Hoston River water	Spanggord *et al.* (1982b)
Hydrolysis	Not significant	Spanggord *et al.* (1982b)
Other Abiotic Reactions		
Aqueous Speciation	Not likely	—
Aqueous Complexation	No reports; probable, but weak	—
Abiotic Reduction	Not under aerobic conditions; perhaps in anaerobic systems	Spanggord *et al.* (1982b)
Polymerization	No reports; perhaps in reduced transformation products (amino compounds)	—
Binding to Soil Solids	No reports; perhaps in amino reduced compounds	—

Table C.3: 2,4,6-trinitrotoluene (TNT) physical, chemical and environmental properties.

CAS Reg. No.: 118-96-7
Other names: α-trinitrotoluene, *sym*-trinitrotoluene,
1-methyl-2,4,6-trinitrobenzene
Formula (semistructural, empirical): $C_6H_2CH_3(NO_2)_3$ or $C_7H_5N_3O_6$
Molecular Mass: 227.13 g·mol^{-1}



Parameter	Value / Comment	Reference
Density	1.654 g·cm^{-3} 1.654 - 1.663 g·cm^{-3}	Dean (1985) Urbanski (1964)
Melting Point	80.1 °C 80.65 °C	Dean (1985) Urbanski (1964)
Crystallography	monoclinic rhombohedra from ethanol: colorless Commercial form: yellow needles or columns	Verschueren (1983) Merck (1983)
Dipole moment	1.37 D	Merck (1983)
Vapor Properties (TNT)		
Henry's Law Constant, K_H	0.18 torr L mol^{-1} < 0.02 torr L mol^{-1} 1.1 E-08 atm·m^3·mol^{-1} [25°C]	Spanggord *et al.* (1980) Haynes & Smith (1981) Rosenblatt *et al.* (1989)
Vapor Pressure (solid)	20.0 °C 1.28 E-06 torr 25 °C 5.51 E-06 torr (est.)	Leggett, Jenkins, Murrmann (1977) Rosenblatt *et al.* (1989)
Aqueous Solubility (TNT)		
0° C	100 mg·L^{-1}	Urbanski (1964)
10° C	110 mg·L^{-1}	Urbanski (1964)
15° C	200 mg·L^{-1} 120 mg·L^{-1}	Verschueren (1983) Urbanski (1964)
20°C	130 mg·L^{-1}	Urbanski (1964)
25°C	~100 mg·L^{-1} (~0.01%) 150 mg·L^{-1}	Merck (1983) Urbanski (1964)
Hygroscopy	Non-hygroscopic; 0.05% water	Urbanski (1964)
EPA Drinking Water Std. (DWEL)	0.020 mg·L^{-1}	[in Rosenblatt *et al.* 1989]
RMCL (Recc. Max. Contam. Lev.)	44 µg·L^{-1}	DOA (1980; Spalding & Fulton, 1988)

TNT Solubility in Organics Solvents [g L^{-1}]

	10° C	15° C	20° C	25° C	
Acetone	780	920	1090	1320	Urbanski (1964)
Benzene	360	500	670	880	"
Toluene	380	450	550	670	"
Ethanol (95%)	8.5	10.7	12.3	14.8	"

(Continued)

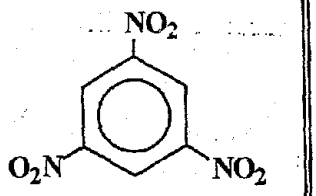
Table C.3: TNT (concluded)

Parameter	*Value / Comment*	*Reference*
Environmental Processes (TNT)		
Partitioning Coefficients	log K_{ow} = 2.06 ; 1.86	Rosenblatt *et al.* (1989); Jenkins (1989)
	log K_{oc} = 2.72	Rosenblatt (1986)
	K_d = 53 ± 20 mL·g^{-1} 2 - 56 mL·g^{-1}	Spanggord *et al.* (1980)
Diffusion	Water: 6.71 E-06 cm^2·s^{-1} (25°C; est.) Air: 0.064 cm^2·s^{-1} (25°C; est.)	Rosenblatt *et al.* (1989)
Biotransformation	Major process in surface and ground waters. Succesive reduction of nitro (R-N[+v]O_2) to amine (R-N[-I]H_2) groups is most common.	Rosenblatt *et al.* (1989)
	A few reports of microbial growth on and mineralization of TNT *via* elimination reactions; these microorganisms use TNT as the sole nitrogen, and carbon sorce.	Boopathy and Kulpa (1992), Duque et al. (1993), Unkefer et al. (1990)
	Half-life in Groundwater (est.): 8640 hrs (12 mo.) to 672 hrs (4 weeks)	Howard *et al.* (1991)Biodegradation
	Aerobic: Major transformation process in surface water; significant in soils as well; slow rates Products: hydroxamino and azoxytoluene compounds	Kaplan and Kaplan (1982) Spanggord *et al.* (1980a)
	Anaerobic: Primarily nitro-to-amino reduction; moderate rates	
Toxicity	EPA possible human carcinogen; May absorb through skin; Can cause headache, weakness, anemia, liver injury; Vapors are toxic	[in Rosenblatt *et al.*, 1989]; Merck (1983)
Photosensitivity	Significant. To: 2,4,6-trinitrobenzaldehyde, 1,3,5-trinitrobenzene, 3,5-dinitroaniline, 2-amino-4,6-dinitrobenzoic acid, azoxydicarboxylic acid	Kay (1980), Burlinson (1980)
Hydrolysis	alkaline sensitive	
Other Abiotic Reactions		
Aqueous Speciation	Not likely	—
Aqueous Complexation	Forms complexes with surfactants.	Kaplan and Kaplan (1982)
Abiotic Reduction	Apparent major reaction pathway; anaerobic or aerobic conditions	—
Polymerization	No reports. Reduction products may form azo or azoxy compounds *via* amino intermediates.	—
Binding to Soil Solids	No reports. Reduction products appear to bind with carboxyl and/or other functional groups in soil organics (by analogy to aniline)	Weber *et al.*, 1992; Wolfe and Macalady, 1992; Bollag *et al.*, 1983; Parris, 1980; Hsu and Bartha, 1974.

Table C.4: 1,3,5-trinitrobenzene (TNB) physical, chemical and environmental properties

CAS Reg. No.: 99-35-4
Other names: *sym*-trinitrobenzene

Formula (semistructural, empirical): $C_6H_3(NO_2)_3$ or $C_6H_3N_3O_6$
Molecular Mass: 213.11 g·mol^{-1}



Parameter	Description	Reference
Density	1.688 (20°C) 1,654-1.663	Dean (1985) Urbanski (1964)
Melting Point	122.5° C 122 ° C	Dean (1985), Merck (1983) Urbanski (1964)
Crystallography	orthorhombic, bipyrimidal plates from glacial acetic acid	Merck (1983)
Dipole moment	~0 (symmetry)	—
Vapor Properties (TNB)		
Henry's Law Constant, K_H	2.21 E-09 atm m^3 mol^{-1}	Rosenblatt *et al.* (1989)
Vapor Pressure (solid)	3.03 E-06 torr (25 °C; est.); Can sublimate with careful heating	Rosenblatt *et al.* (1989); Merck (1983)
Aqueous Solubility (TNB)		
15° C 20° C ~25° C 25° C	– – ~350 mg·L^{-1} (0.035 g / 100 g-water) 385 mg·L^{-1}	 Merck (1983); Rosenblatt et al. (1989)
Solubility in Organics (TNB)		
Acetone Benzene Toluene Ethanol (95%)	– 6 200 mg L^{-1} – 1 900 mg L^{-1}	 Merck (1983) Merck (1983)
Environmental Processes (TNB)		
Partitioning Coefficients	K_d: log K_{ow}: 1.18 log K_{oc}: 1.30 (est.)	 Hansch and Leo (1979) Rosenblatt *et al.* (1989)
Diffusion Coefficient	Water: 7.20 E-06 cm s^{-1} Air: 0.068 cm s^{-1}	Rosenblatt *et al.* (1989)
Biodegradation	Probable. Similar to TNT, reduction and perhaps elimination of the nitro group.	–
Toxicity	–	–
Photosensitivity	TNB is photostable. TNB is one phototransformation product of TNT in natural waters.	Rosenblatt *et al.* (1989); Burlinson (1980)

(continued)

Table C.4: TNB (continued)

Parameter	*Description*	*Reference*
	Environmental Processes (TNB) (continued)	
Hydrolysis	Reacts with alkalis readily	Urbanski [1964]
Other Abiotic Reactions		
Aqueous Speciation	Not likely	–
Aqueous Complexation	May form complexes with surfactants (analogy to TNT).	–
Abiotic Reduction	No data. Probably a significant reaction pathway; anaerobic or aerobic conditions	–
Polymerization	No reports. Reduction products may form azo or azoxy compounds *via* amino intermediates.	–
Binding to Soil Solids	No reports. Reduction products could bind with carboxyl and/or other functional groups in soil organics (by analogy to aniline)	–